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L13: Entry 23 of 38 File: USPT Oct 17, 2000

DOCUMENT-IDENTIFIER: US 6133026 A
TITLE: Condensed plasmid-liposome complex for transfection

Abstract Paragraph Left (1):
A plasmid-liposome composition for transfection of a cell is described. The composition includes plasmid molecules condensed with a polycationic condensing agent and cationic liposomes. Also disclosed is a method for preparing the plasmid-liposome complexes.

Brief Summary Paragraph Right (7):
In one aspect, the invention includes a composition of plasmid-liposome complexes for use in transfecting a host cell with a gene contained in a plasmid. The composition includes plasmid molecules that are condensed with a polycationic condensing agent and suspended in a low-ionic strength aqueous medium and cationic liposomes formed of a cationic vesicle-forming lipid. The complexes have a ratio of liposome lipid to plasmid of greater than 5 nmole liposome lipid/.mu.g plasmid and less than 25 nmole liposome lipid/.mu.g plasmid and have a substantially homogeneous size of less than about 200 nm.

Brief Summary Paragraph Right (12):
The cationic liposomes are composed of a cationic vesicle-forming lipid selected from dimethyldioctadecylammonium (DDAB), 1,2-diolelyloxy-3-(trimethylamino) propane (DOTAP), N-[1-(2,3,-ditetradecyloxy)propyl]-N,N-dimethyl-N-hydroxyethylammonium bromide (DMRIE), N-[1-(2,3,-dioleyloxy)propyl]-N,N-dimethyl-N-hydroxy ethylammonium bromide (DORIE), N-[1-(2,3-dioleyloxy) propyl]-N,N,N-trimethylammonium chloride (DOTMA), and 3.beta.[N-(N',N'-dimethylaminoethane) carbamoly] cholesterol (DC-Chol).

Brief Summary Paragraph Right (15):
In yet another embodiment, the cationic liposomes have a surface coating of hydrophilic polymer chains by including a vesicle-forming lipid derivatized with such a hydrophilic polymer. In one embodiment, at least a portion of the hydrophilic polymer chains are joined to the vesicle-forming lipid by a releasable bond, e.g., a bond effective to release the hydrophilic polymer chains in response to an existing or an induced physiologic condition. The plasmid-liposome complexes can further include a ligand attached to distal ends of the hydrophilic polymer chains for ligand-specific binding to a receptor molecule on a target cell surface. For example, and in a preferred embodiment, the hydrophilic polymer is polyethyleneglycol.

Brief Summary Paragraph Right (18):
In a preferred embodiment, the plasmid-liposome complex is for use in transfecting a host cell in the lung of a subject with a DNA plasmid containing cystic fibrosis transmembrane conductance regulator or, for lung carcinomas, cytokines, such as interleukin-2, or tumor suppressor genes, such as p53.

Drawing Description Paragraph Right (5):
FIG. 5 is a plot of particle diameter, in nm as measured by dynamic light scattering, as a function of storage time at 4 C, for plasmid-liposome complexes of the invention;

Drawing Description Paragraph Right (16):
FIG. 16 is a plot showing luciferase expression, in ng/mg protein, in various mice tissues 24 hours after administration of plasmid-liposome complexes prepared with pNSL plasmid encoding for luciferase to tumor-bearing mice (metastasis model of Lewis Lung Tumor).

Detailed Description Paragraph Right (5):
Another cationic vesicle-forming lipid which may be employed is cholesterol amine and

related cationic sterols. Exemplary cationic lipids include 1,2-diolelyloxy-3-(trimethylamino) propane (DOTAP); N-[1-(2,3,-ditetradecyloxy)propyl]-N,N-dimethyl-N-hydroxyethylammonium bromide (DMRIE); N-[1-(2,3,-dioleyloxy)propyl]-N,N-dimethyl-N-hydroxy ethylammonium bromide (DORIE); N-[1-(2,3-dioleyloxy) propyl]-N,N,N-trimethylammonium chloride (DOTMA); 3.beta.[N-(N',N'-dimethylaminoethane) carbamoly] cholesterol (DC-Chol); and dimethyldioctadecylammonium (DDAB).

Detailed Description Paragraph Right (12):
Other releasable linkages include pH sensitive bonds and bonds which are cleaved upon exposure to glucose, light or heat. By way of an example, the hydrophilic polymer chains can be attached to the liposome by a pH sensitive bond, and the plasmid-liposome complexes are targeted to a site having a pH effective to cleave the bond and release the hydrophilic chains, such as a tumor region. Exemplary pH sensitive bonds include acyloxyalkyl ether, acetal and ketal bonds. Another example is where the cleavable bond is a disulfide bond, broadly intended herein to refer to sulfur-containing bonds. Sulfur-containing bonds can be synthesized to achieve a selected degree of lability and include disulfide bonds, mixed sulfide-sulfone bonds and sulfide-sulfoxide bonds. Of the three bonds, the disulfide bond is least susceptible to thiolysis and the sulfide-sulfoxide bond is most susceptible.

Detailed Description Paragraph Right (13):
Such releasable bonds are useful to tailor the rate of release of the hydrophilic polymer segment from the plasmid-liposome complexes. For example, a very labile disulfide bond can be used for targeting to blood cells or endothelial cells, since these cells are readily accessible and a shorter liposome blood circulation lifetime is sufficient. At the other extreme, a long-lasting or hearty disulfide bond can be used when the target is tumor tissue or other organs where a longer liposome blood circulation lifetime is generally needed for the complexes to reach the desired target.

Detailed Description Paragraph Right (14):
The releasable bond attaching the hydrophilic polymer chains to the liposome is cleaved in vivo typically as a result of change in environment, such as when the liposomes reach a specific site with a slightly lower pH, such as a region of tumor tissue, or a site with reducing conditions, such as a hypoxic tumor. Reducing conditions in vivo can also be effected by administration of a reducing agent, such as ascorbate, cysteine or glutathione. The cleavable bond may also be broken in response to an external stimuli, such as light or heat.

Detailed Description Paragraph Right (15):
In another embodiment, the plasmid-liposome complexes include an affinity moiety or targeting ligand effective to bind specifically to target cells at which the therapy is aimed. Such moieties can be attached to the surface of the liposome or to the distal ends of hydrophilic polymer chains. Exemplary moieties include antibodies, ligands for specific binding to target cell surface receptors and the like, as described, for example, in PCT application Nos. WO US94/03103, WO 98/16202 and WO 98/16201. The moiety can also be a hydrophobic segment to facilitate fusion of the complex with a target cell.

Detailed Description Paragraph Right (23):
In the second class are polypeptides designed to treat any existing pathology, such as cancer, or a pathogenic condition such as viral infection. Examples include gene therapy to supply the p53 gene for cancer therapy, the gene for the CD4 peptide to inhibit HIV infection, the gene for the Pseudomonas peptide to inhibit binding of Pseudomonas to epithelial cells, and specific antibody genes to inhibit a targeted pathogen.

Detailed Description Paragraph Right (24):
The third class includes genes intended to produce an mRNA transcript that can act as an antisense molecule to inhibit an undesirable protein expression, such as overexpression of proteins specific for tumor growth, or expression of viral proteins.

Detailed Description Paragraph Right (29):
FIG. 1 is a computer-generated image of a negative-stain transmission electron micrograph of a luciferase-encoding pNSL plasmid condensed with total histone, prepared as described in Example 1. As seen, the plasmid is condensed into discrete, single particles of about 100 nm in diameter and less.

Detailed Description Paragraph Right (52):
In another study performed in support of the invention, detailed in Example 3, mice bearing metastatic Lewis lung tumors were treated with plasmid-liposome complexes. After tumor inoculation, the mice were treated with one of eight treatment regimens set forth in Table 5 in Example 3. The treatments included administration of plasmid-liposomes complexes prepared using plasmids carrying genes encoding for p53 (pCMVp53) and interleukin 2 (pCMVIL2) and a combination treatment of ganciclovir and plasmid-liposome complexes prepared with a pHSVtk (herpes Simplex Virus thymidine kinase) plasmid. A control group of animals received saline, and comparative groups of animals received complexes prepared with the pNSL plasmid encoding for luciferease (Example 1) or ganciclovir alone.

Detailed Description Paragraph Right (53):
FIGS. 14A-14C show the percent of surviving animals as a function of days post tumor-cell inoculation. In all of FIGS. 14A-14C, the animals treated with saline are represented with the closed squares. As seen, all of the saline-treated animals died by 24 days after tumor inoculation. In FIG. 14A, the animals treated with complexes including the 50 .mu.g (solid inverted triangles) and 75 .mu.g (open squares) pHSVtk plasmid and with ganciclovir are shown. All of the animals treated with ganciclovir alone (open circles) died by day 37. In contrast, the animals treated with the combination therapy of liposome-plasmid complexes and ganciclovir fared better, with 70% of those treated with the higher plasmid dose surviving the study.

Detailed Description Paragraph Right (57):
In another study using the Lewis lung tumor model, the pNSL-luciferase-plasmid-liposome complex was administered to mice and luciferase expression in various tissues was examined 24 hours after administration of plasmid-liposome complexes. As seen in FIG. 16, the highest luciferase expression was observed in the lung and in the tumors.

Detailed Description Paragraph Right (62):
Dimethyldioctadecylammonium (DDAB) was purchased from Avanti Polar Lipids, Inc. (Birmingham, Ala.). Cholesterol, greater than 99% pure, was obtained from Nu-Chek (Elysian, Minn.).

Detailed Description Paragraph Right (67):
Size distribution measurements were obtained by dynamic light scattering (DLS) using a Coulter N4MD instrument, operated according to the manufacturer's, instructions. The results were expressed as the mean diameter in nm and standard deviation of a Gaussian distribution of particles by relative volume.

Detailed Description Paragraph Right (72):
Cationic liposomes were prepared according to standard procedures by dissolving 10 .mu.mol DDAB and 10 .mu.mol cholesterol in an organic solvent containing primarily CHCl.sub.3. The lipids were dried to a thin film by rotation under reduced pressure. The lipid film was hydrated by addition of distilled water to form a suspension of liposomes at a concentration of 20 .mu.mole/ml. The liposomes were sized by sonication or by sequential extrusion through Nucleopore polycarbonate membranes with pore sizes of 0.4 .mu.m, 0.2 .mu.m, 0.1 .mu.m and 0.05 .mu.m to obtain liposomes of less than about 200 nm in size (Nucleopore, Pleasanton, Calif.).

Detailed Description Paragraph Right (81):
B. Tumor Inoculation

Detailed Description Paragraph Right (82):
Eighty B6C3-F1 male mice were obtained from Taconic Farms (German Town, N.Y.) and allowed to acclimate for 3 days prior to initiation of the experiment. Animals were housed in appropriate isolated caging with ad lib sterile rodent food and acidified water and a 12:12 light:dark cycle. Animals were randomized into treatment groups prior to inoculation of tumors based on body weight. Animals were randomized into treatment groups prior to inoculation of tumors.

Detailed Description Paragraph Right (83):
All the animals were observed daily for general well-being. The animals were weighed prior to inoculation of tumor cells and twice weekly thereafter. Animals observed to have a 15% or greater weight loss from their starting weight, or any animal in distress, was euthanized and examined for the presence and size of metastatic foci in the lung, liver and spleen.

Detailed Description Paragraph Right (84):

Tumors were inoculated by taking growing Lewis lung tumors from other B6C3-F1 mice by sterile surgical harvest after euthanasia. The tumors were mechanically minced as finely as possible and briefly digested in an enzyme mix of collagenase, protease and DNAse at 37.degree. C. After digestion and washing in media (RPMI+15% FCS) cells were counted with a hemocytometer. Cells were spun down and resuspended in media at 10.sup.6 cells per ml (10.sup.5 cells per 0.1 ml injection). The resuspended cells were drawn into individual syringes (0.1 ml, with continuous mixing) for intravenous injection into the tail vein of each animal.

Detailed Description Paragraph Right (86):
Animals were treated, beginning 3 days after inoculation with tumor cells, with one of the eight regimens set forth in Table 5. The 10 animals in each group were treated 5 times at one-week intervals, except as indicated for the ganciclovir treatment group no. 30 and for the animals receiving

Detailed Description Paragraph Right (88):
Twenty-four hours after the last treatment, surviving animals were euthanized for tissue and tumor harvest and examination. The percent of surviving animals for each treatment group are shown in FIGS. 14A-14C. Toxicity of formulation no. 34 is shown in FIG. 15 and luciferase expression of formulation no. 34 is shown in FIG. 16.

Detailed Description Paragraph Center (7):
In vivo Transfection Tumor-Bearing Mice

CLAIMS:

8. The composition of claim 1, wherein the cationic liposomes are composed of a cationic vesicle-forming lipid selected from the group consisting of dimethyldioctadecylammonium (DDAB), 1,2-diolelyloxy-3-(trimethylamino) propane (DOTAP), N-[1-(2,3 ,-ditetradecyloxy)propyl]-N,N-dimethyl-N-hydroxyethylammonium bromide (DMRIE), N-[1-(2,3,-dioleyloxy)propyl]-N,N-dimethyl-N-hydroxy ethylammonium bromide (DORIE), N-[1-(2,3-dioleyloxy) propyl]-N,N,N-trimethylammonium chloride (DOTMA), and 3.beta.[N-(N',N'-dimethylaminoethane) carbamoly] cholesterol (DC-Chol).

13. The composition of claim 11, wherein the plasmid-liposome complexes further include a ligand attached to distal ends of the hydrophilic polymer chains for ligand-specific binding to a receptor molecule on a target cell surface.

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End of Result Set

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L14: Entry 1 of 1 File: USPT Jun 20, 2000

US-PAT-NO: 6077834
DOCUMENT-IDENTIFIER: US 6077834 A

TITLE: Receptor ligand-facilitated delivery of biologically active molecules

DATE-ISSUED: June 20, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Cheng; Pi-Wan	Omaha	NE	68118	

APPL-NO: 8/ 790290 [PALM]
DATE FILED: January 29, 1997

PARENT-CASE:
CROSS-REFERENCE TO RELATED APPLICATION This application is based on United States Provisional Application Ser. No. 60/011,368 filed Feb. 9, 1996, entitled RECEPTOR LIGAND-FACILITATED GENE TRANSFER. Priority is claimed under 35 U.S.C. Section 119(e).

INT-CL: [7] A61 K 48/00

US-CL-ISSUED: 514/44; 424/450, 435/69.1, 435/320.1, 435/325, 435/455, 435/458
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FIELD-OF-SEARCH: 424/450, 514/7.1, 514/9, 514/44, 435/69.1, 435/172.3, 435/325, 435/320.1, 435/455, 435/458, 935/52, 935/66, 935/32, 935/57

PRIOR-ART-DISCLOSED:

U.S. PATENT DOCUMENTS

Search Selected Search ALL

PAT-NO	ISSUE-DATE	PATENTEE-NAME	US-CL
4897355	January 1990	Eppstein et al.	435/325
5264618	November 1993	Felgner et al.	
5279833	January 1994	Rose	424/450
5328470	July 1994	Nabel et al.	604/101
5334761	August 1994	Gebeyehu et al.	564/197
5451661	September 1995	Wan	530/345
5455157	October 1995	Hinzpeter et al.	435/6
5459127	October 1995	Felgner et al.	514/7
5547932	August 1996	Curiel et al.	435/65
5578475	November 1996	Jessee	435/455
5580859	December 1996	Felgnel et al.	514/44
5631237	May 1997	Dzau et al.	514/44
5635380	June 1997	Naftelan et al.	435/455
5681571	October 1997	Holmgren et al.	435/236.1
5736392	April 1998	Hawley-Nelson et al.	435/320.1
5780052	July 1998	Khaw et al.	424/450
5786214	July 1998	Holmberg	435/375
5827703	October 1998	Debs et al.	435/455
5837533	November 1998	Boutin	435/320.1

FOREIGN PATENT DOCUMENTS

FOREIGN-PAT-NO	PUBN-DATE	COUNTRY	US-CL
WO 95/17373	June 1995	WOX	

OTHER PUBLICATIONS

Webster's II New Riverside University Dictionary (1994, pp. 132 and 299).
Coghlan, New Scientist, vol. 148, 14-15, 1995.
Mastrangelo et al., Seminars in Oncology, vol. 25, 1:4-21, 1996.
Gunzberg et al., Molecular Medicine Today, 1995, pp. 410-417.
Hong et al., Chinese Medical J., 108, 5, 332-337, 1995.
Wagner et al., P. N. A. S., vol. 89, 6099-6103, 1992.
Kolata, New York Times, Jul. 25, 1995, p. 3, C3, 1995.
Gao et al., J. of Liposome Res., 3, 1, 17-30, 1993.
Cheng, P.W., "Correction of the Chloride Transport Abnormality of CFT1 Cells by Transferrin-Facilitated Gene Transfer Mediated by Liposome", Abstract Submitted Sep. 8, 1995 for the Ninth Annual North American Cystic Fibrosis Conference, Dallas, Texas--Oct. 12-15, 1995.
Cheng, Pi-Wan, "Receptor Ligand-Facilitated Gene Transfer: Enhancement of Liposome-Mediated Gene Transfer and Expression by Transferrin", Human Gene Therapy, (Feb. 10, 1996) 7:275-282, Mary Ann Liebert, Inc.

ART-UNIT: 163

PRIMARY-EXAMINER: Priebe; Scott D.

ASSISTANT-EXAMINER: Nguyen; Dave Trong

ATTY-AGENT-FIRM: Zarley, McKee, Thomte, Voorhees & Sease

ABSTRACT:

Disclosed is a delivery system for biologically active molecules or agents which must enter cells to exert their effect. The delivery system comprises a mixture of cationic lipid in combination with a receptor ligand and is particularly suited for intracellular delivery of polynucleotides.

19 Claims, 22 Drawing figures
Exemplary Claim Number: 1
Number of Drawing Sheets: 15

GOVT-INTEREST:

This invention was supported at least in part by a grant from the National Institutes of Health (HL48282). The United States Government has certain rights in this invention.

BRIEF SUMMARY:

FIELD OF THE INVENTION

This invention is in the field of intracellular delivery of biologically active molecules.

BACKGROUND OF THE INVENTION

At the present time, major somatic gene transfer approaches employ either viral (Morgan, J. R., Tompkins, R. G., and Yarmuch, M. L. (1993), "Advances in recombinant retroviruses for gene delivery," Adv. Drug Del., Rev. 12, 143-158; Colledge, W. H. (1994), "Cystic fibrosis gene therapy." Curr. Opin. Gene Develop., 4, 466-471; Trapnell, B. C. and Gorziglia, M. (1994), "Gene therapy using adenoviral vectors," Curr. Opin. Biotechnol., 5, 617-625) or nonviral vectors (Cotten, M. and Wagner, E. (1993), "Non-viral approaches to gene therapy," Curr. Opin. Biotech., 4, 705-710; Ledley, F. D. (1994), "Non-viral gene therapy," Curr. Opin. Biotechnol., 5, 626-636).

Viral vector-directed gene transfer shows high gene transfer efficiency but is deficient in several areas. For example, some viral vectors randomly integrate DNA into host genomes (Olsen, J. C., Huang, W., Johnson, L. G., and Boucher, R. C. (1994) "Persistence of adenoviral vector gene expression in CF airway cells is due to integration of vector sequences into chromosomal DNA," Pediatr. Pulm. S10, 230; Russel, D. W., Miller, A. D., and Alexander, I. E. (1994) "Adeno-associated virus vectors preferentially transduce cells in S phase," Proc. Natl. Acad. Sci. 91, 8915-8919) posing potential risks, including neoplastic transformation (Colledge et al. (1994) supra; Fairbairn, L. J., Cross, M. A. and Arrand, J. R. (1994) "Paterson Symposium 1993-Gene therapy," Brit. J. Cancer, 59, 972-975). In addition, adenoviral vectors induce host inflammatory and immune responses, rendering these vectors ineffective in repeated application (Ginsburg, H. S., Moldawer, L. L., Schgal, P. B., Redimgton, M., Kilian, D. L., Chanock, R. M., and Prince, G. A. (1991), "A mouse model for investigating the molecular pathogenesis of adenovirus pneumonia," Proc. Natl. Acad. Sci. USA 88, 1651-1655; Yang, Y. P., Nunes, F. A., Berencsi, K., Gonczol, E., Engelhardt, J. F., and Wilson, J. M. (1994), "Inactivation of E2a in recombinant adenovirus improves the prospect for gene therapy in cystic fibrosis," Nature Genetics 7, 362-369; Yei, S., Mittereder, N., Tany, K., O'Sullivan, C., and Trapnell, B. C. (1994) "Adenovirus-mediated gene transfer for cystic fibrosis: Quantitative evaluation of repeated in vivo vector administration to the lung," Gene Therapy 1, 192-200; Trapnell et al. (1994), supra). Retroviral vectors require dividing cells for stable integration (Miller, D. C., Adam, M. A., and Miller, A. D. (1990), "Gene transfer by retrovirus vectors occurs only in cells that are actively replicating at the time of infection," Mol. Cell Biol. 10, 4239-4242), making these vectors unsuitable for gene therapy of terminally differentiated cells. Similarly, for efficient transduction, adeno-associated virus prefers cells in the S phase to the cells in stationary culture (Russel et al. (1994) supra). Furthermore, the requirement of adenovirus and high multiplicity of infection of adeno-associated virus for efficient transduction (Russel et al., (1994) supra) coupled with difficulty in obtaining virus preparations with high titer has limited the use of this virus as a routine gene therapy vector.

Some of the problems associated with using these viral vectors can be circumvented using gene transfer agents, such as molecular conjugates (Wu, G. Y. and Wu, C. H. (1987) "Receptor-mediated in vitro gene transformation by a soluble DNA carrier system," J. Biol. Chem. 262, 4429-4432; Wagner, E., Zenke, M., Cotten, M., Beug, H., and Birnstiel, M. L., (1990) "Transferrin-polycation conjugates as carriers for DNA uptake into cells," Proc. Natl. Acad. Sci., USA 87, 3410-3414; Findeis, M. A., Merwin, J. J., Spitalny, G. L., and Chiou, H. C. (1993), "Targeted delivery of DNA for gene therapy via receptors," TIBTECH 11, 202-205; Ferkol, T., Kaetzel, C. S., and Davis, P. B. (1993), "Gene transfer into respiratory epithelial cells by targeting the polymeric immunoglobulin receptor," J. Clin. Invest. 92, 2394-2400; Monsigny, M., Roche, A.-C., Midous, P., and Mayer, R. (1994) "Glycoconjugates as carriers for specific delivery of therapeutic drugs and genes," Adv. Drug Del. Rev. 4, 1-24; Yin, W. and Cheng, P-W. (1994), "Lectin conjugate-directed gene transfer to airway epithelial cells," Biochem. Biophys. Res. Commun., 205, 826-833) and cationic liposomes (Felgner, J. H., Gadek, T. R., Holm, M., Roman, R., Chan, H. W., Wenz, M., Northro, J. P., Ringold, G. M., and Danielsen, M. (1987) "Lipofectin: A highly efficient, lipid-mediated DNA-transfection procedure," Proc. Natl. Acad. Sci., USA 84: 7413-7417). Molecular conjugates are prepared by chemically linking receptor ligands with polycations (Wagner, E., Curiel, D., and Cotten, M. (1994), "Delivery of drugs, proteins and genes into cells using transferrin as a ligand for receptor-mediated endocytosis," Adv. Drug Del., Rev. 14, 113-135). Molecular conjugates for receptor-mediated gene delivery can also be prepared by chemically linking antibodies or fragments thereof with polycations (Cotten, M. and Wagner, E. (1993) supra; references therein and Ferkol, T. et al. (1993) supra). The polycations serve as carriers of DNA while the ligands target the receptors on cell surfaces. Upon binding to the receptors, the conjugates along with the DNA are internalized via receptor-mediated endocytosis (Findeis (1993) supra; Wagner (1994) supra; Curiel, D. T. (1994), "High-efficiency gene transfer employing adenovirus-polylysine-DNA complex," Nat. Immun., 13, 141-164).

In cationic liposome-mediated gene transfer, liposomes bind to DNA via ionic interaction and liposomes facilitate the delivery of DNA presumably by fusion with the plasma membrane (Felgner et al. (1987) supra) and/or endocytosis (Zhou, X. and Huang, L., (1994) "DNA transfection mediated by cationic liposomes containing lipopolylysine: characterization and mechanism of action," Biochem. Biophys. Acta., 1189, 195-203. These agents are easy to prepare, can deliver DNA of any size (Wagner et al. (1994) supra), but generally suffer from low transfection efficiency (Colledge (1994) supra).

SUMMARY OF THE INVENTION

This invention employs a delivery system for biologically active molecules which must enter cells to exert their biological effect. Such molecules include but are not limited to peptides, proteins, or polynucleotides such as DNA or RNA. In a preferred embodiment the molecule is a gene for transfection in gene therapy protocal. The delivery system is a mixture composed of a cationic lipid which is preferably in a liposome formulation and a receptor ligand, such as transferrin, wherein the ligand is not covalently bound to a liposome component. The receptor ligand is first added to the cationic liposome formulation and incubated. The biologically active agent to be delivered to cells is then added to the resulting ligand-liposome combination and the mixture is again incubated. The order in which the components are combined, i.e. ligand with liposome formulation followed by nucleic acid, is critical to obtaining high efficiency transfection.

Generally, the methods of this invention can employ any ligand having an affinity for a cell surface receptor. The term ligand is used broadly herein and includes receptor ligands such as transferrin, insulin, cholera toxin, adenovirus fiber KNOB peptide, as well as antibodies and antibody fragments (e.g. Fab fragments) to receptors, such as anti-secretory components, and peptides and proteins, such as epidermal growth factor and viral proteins, particularly those viral proteins which the receptor-mediated endocytosis mechanism stimulates. The ligand employed can be one, such as transferrin or insulin, that targets a wide range of cell types or one that targets a specific cell type.

Generally, the methods of this invention can employ any mono-or polycationic lipid and neutral lipid in a cationic liposome formulation. Cationic lipids including DOTMA, DDAB, DOSPA, DORI, DORI-ester, DORI-ether, DMRIE, DOTAP, TM-TPS and cationic lipids structurally related thereto. [Definitions for each of these acronyms are provided herein and are well-known in the art.]

More specifically, this invention provides transfection agents in which the <u>ligand</u> is

transferrin, insulin, cholera toxin or adenovirus fiber KNOB peptide, and in which the cationic liposome formulation comprises the cationic lipids DOTMA (N-[1-(2,3-dioleyloxy)propyl]-N,N,N-trimethyl), or DDAB (dimethyl dioctadecylammonimum bromide) and an appropriate neutral lipid. Useful cationic liposome formulations include those in which the neutral lipid is DOPE (dioleoyl phosphatidylethanolamine). Preferred cationic liposome formulations are "lipofectin" (Trademark) which is a commercially available 1:1 liposome formulation of DOTMA and DOPE and "lipofectace" (Trademark) which is a commercially available 1:2.5 liposome formulation of DDAB and DOPE.

Certain fractions and/or components of serum have been found to enhance transfection efficiency of cationic liposomes. These fractions or components, when used in place of receptor ligands in the protocols of this invention, enhance liposome transfection efficiency.

The method of this invention is a high-efficiency gene transfer method which employs transfection reagents that are easy to prepare. In exemplified embodiments the reagent ingredients are commercially available. Further, receptor ligands prepared from one animal source may be employed for gene therapy in the same animal species, thus mitigating or preventing host inflammatory and immune responses which have been a major drawback for human gene therapy employing adenoviral vectors. Liposomes appear to cause little or no apparent host inflammatory and immune response. Utilization of human transferrin or other human ligands in combination with cationic liposomes can circumvent the host immune response while achieving high gene transfer efficiency in humans.

The methods described herein with "lipofectin" and transferrin can yield 100% transfection efficiency in HeLa cells. The "lipofectin"-transferrin transfection protocol of this invention can also be used to correct the chloride conductance abnormality in immortalized CF airway epithelial cells (CFT1) by delivery of CFTR CDNA. Transferrin also significantly enhances transfection by "lipofectace" of HeLa cells.

The transfection agents and methods of this invention are useful in in vitro and in vivo transfection applications. The simplicity of the formulation and high transfection efficiency by these reagents facilitate the development of suitable transfection reagents for human gene therapy.

DRAWING DESCRIPTION:

BRIEF DESCRIPTION OF THE FIGURES

FIGS. 1(a)-(h) illustrates X-Gal staining of HeLa cells transfected with transferrin and "lipofectin" containing either 16 or 0 .mu.g transferrin (F0) (1a), and the transfected cells of six consecutive passages (F1 to F6)(1b-1g). Cells transfected with "lipofectin"-DNA, i.e. 0 .mu.g transferrin, served as the controls (C)(1h). For each set of F0, cells in 9 wells were transfected. After culturing for 48 hours following transfection, cells in 3 wells were stained with X-gal, cells in another 3 wells were assayed for .beta.-galactosidase activity, and cells in the remaining 3 wells were subcultured to 9 wells and employed for subsequent study. The subcultured cells were processed as described below but without transfection. Only F0(C) (1h) is shown because F1(C) and F2(C) are similar to F5(1f), and F3(C), F4(C), F5(C), and F6(C) are similar to F6(1g). A detailed experimental protocol is described in the Examples.

FIG. 2 illustrates enhancement of DNA transfer by transferrin in the presence of "lipofectin". The symbols .box-solid., .circle-solid., .quadrature., and .largecircle. represent .sup.35 S-pCMVlacZ transferred to the cells with DNA, DNA+transferrin, DNA+"lipofectin", and DNA+ "lipofectin"+transferrin, respectively. The value at each time point was the average of two measurements.

FIG. 3 illustrates Sepharose 4B chromatography of the mixture of (A) [.sup.125 I]transferrin, "lipofectin", and DNA, (B) "lipofectin", DNA, and [.sup.125]transferrin, (C) "lipofectin" and [.sup.125 I]transferrin, and (D) DNA and [.sup.125 I]transferrin. Each component of the mixtures was added sequentially followed by a 15 min incubation period. The % of radioactivity detected in the void volume is indicated.

FIG. 4 illustrates the proposed scheme for the formation of efficient and inefficient gene transfer complexes employing the gene transfer vector composed of transferrin and "lipofectin". These complexes are designated as: (A) "lipofectin"-transferrin, (B) "lipofectin"-DNA-transferrin, (C) "lipofectin"-DNA, and (D) transferrin-DNA. (C) and

(D) are inefficient gene transfer complexes while (B) is the most efficient gene transfer complex. The relative strength of interaction between each pair of components are: "lipofectin"-DNA>>transferrin-DNA>>transferrin-"lipofectin".

FIG. 5 is a graph of .beta.-galactosidase activity and % blue cells as a function of concentration of transferrin illustrating transferrin enhancement of transfection efficiency of "lipofectin"--mediated gene (pCMVlacZ) transfer.

FIG. 6 is a graph illustrating the correction of the chloride ion efflux defect in CFT1 cells. Percent .sup.36 Cl-remaining is plotted as a function of time. In the graph, cells transfected with transferrin, "lipofectin" and pCMVCFTR are indicated by an open diamond (.diamond.) and cells transfected with "lipofectin" and the DNA are indicated by open squares (.quadrature.). In a separate experiment, the results of which are not shown, the same results as found for transfection with pCMVlacZ plus "lipofectin" were found for CFT1 cells without transfection, CFTI cells transfected with transferrin DNA and "lipofectin" (added in that order) and CFT1 cells transfected with transferrin plus DNA.

FIG. 7 is a graph similar to that of FIG. 6 plotting percent .sup.36 Cl.sup.- remaining as a function of time in transfected cells. Open circles (.largecircle.) indicate the control (CFT1 cells transfected with "lipofectin"); closed circles (.circle-solid.), open squares (.quadrature.) and closed diamonds (.diamond-solid.) indicate the results for cells transfected with cholera toxin, insulin or transferrin, respectively in combination with "lipofectin" and pCMVCFTR. Cells were preloaded with Na.sup.36 Cl in Cl.sup.- containing medium. Media were collected and replenished with fresh Cl.sup.- -free medium with 0.1 mm ameloride at 1 min intervals. At the end of 3 min, 10 .mu..mu. forskolin was added to the media.

FIGS. 8a and 8b are graphs illustrating transfection efficiency of pCMVlacZ into HeLa cells or CFT1 cells, respectively, as a function of increasing amounts of insulin. The transfection agent is insulin plus "lipofectin".

FIGS. 9a and 9b are graphs illustrating transfection efficiency of pCMVlacZ into HeLa or CFT1 cells, respectively, as a function of cholera toxin concentration. The transfection agent is cholera toxin plus "lipofectin".

FIG. 10 is a graph illustrating transfection efficiency of a "lipofectin" transfection of pCMVlacZ into HeLa cells as a function of serum volume used in place of the receptor ligand.

FIGS. 11a and 11b are graphs illustrating transfection efficiency of a "lipofectin" transfection of pCMVlacZ into HeLa cells in which protein A fractions or con A fractions, respectively, are used to replace receptor ligand.

FIG. 12 is a graph illustrating transfection efficiency of a "lipofectin" transfection using size-fractionated serum samples to replace the receptor ligand.

DETAILED DESCRIPTION:

DETAILED DESCRIPTION OF THE INVENTION

The present invention is in part based on the observation of 98-100% transfection of HeLa cells employing the cationic liposome formulation "lipofectin" and the wide cell range receptor ligand transferrin to deliver an expressible .beta.-galactosidase gene. By comparison simple lipofection using "lipofectin" alone gave low transfection efficiency (3-4%) of the same DNA construct into HeLa cells. The .beta.-galactosidase DNA construct alone or in combination with transferrin gave even lower

transfection efficiencies with HeLa cells (<0.01%), even though the DNA could form a complex with transferrin.

The order of combining the components of the transfection composition was critical to transfection efficiency. The cationic liposome formulation is prepared by conventional methods. The receptor ligand is added to the liposome formulation and the mixture is incubated at room temperature to allow equilibration of complex formation. The nucleic acid, e.g., DNA construct, to be delivered is then added and the mixture incubated a second time at room temperature to allow equilibration of complex formation. Addition of the nucleic acid to the cationic liposome formulation followed by addition of transferrin does not give a high efficiency transfection agent. Likewise, simultaneous

combination of nucleic acid, liposome formulation and receptor ligand does not give a high efficiency transfection agent.

Transferrin receptor-mediated endocytosis is a normal physiological process by which transferrin delivers iron to the cells (Hueber, H. A. and Finch, C. A., (1987) "The physiology of transferrin and transferrin receptors," Physiol. Rev. 67, 520-582). The process entails initial binding of holotransferrin to its receptor on the cell surface at neutral pH (Aisen, P. (1994), "The transferrin receptor and the release of iron from transferrin," Adv. Exp. Med. Biol., 356, 31-40). The transferrin-receptor complex is then internalized to form a clathrin-coated vesicle. Following the release of bound iron as acidic pH in the endosome, the transferrin, still complexed to the receptors, escapes from the endosome and returns to the cell surface where it is released into the circulation. This efficient receptor recycling process has been exploited to deliver foreign DNA to the cells by employing transferrin-polycation conjugates (Wagner et al., 1987; Cotten and Wagner (1993) supra; Wagner et al. (1994) supra; Yin and Cheng, (1994) supra). However, these molecular conjugates have low transfection efficiency (Colledge, (1994) supra; Yin and Cheng, (1994) supra).

The reason for the dramatic enhancement of transfection efficiency observed when transferrin is included in the cationic liposome transfection reagent, but not covalently bound to a liposome component, is not entirely clear at the present time. The role of the cationic liposome in transfection has been thought to be to bind to the negatively charged groups on the cell surface and to fuse with the plasma membrane. During this process, the DNA (and any other species) carried by the cationic liposome gained entrance to the cells (Felgner et al. (1987) supra). An alternative proposal is that the cationic liposome brought the DNA to the cells via the endocytosis pathway (Zhou and Huang (1994) supra).

In the gene transfer method of this invention, transferrin in combination with liposomes facilitates the entry of DNA into the cells at a level twice that of liposomes without transferrin. Although not wishing to be bound by any particular theory of action, high-efficiency gene transfer is believed to be mediated by "lipofectin"-DNA-transferrin complex B as depicted in FIG. 4. The proposed scheme for the formation of effective and ineffective gene transfer complexes illustrated in FIG. 4 is based on the following observations:

1) "Lipofectin" readily forms a complex with a polynucleotide such as DNA through charge-charge interactions and this type of interaction is strong enough to survive electrophoresis conditions. Gershon, H. Ghirlando, R. Guttman, S. B. and Minsky, A. (1993) "Mode of formation and structural features of DNA-cationic liposome complexes used for transfection" Biochemistry 32:7143-7151 reported that interaction of "lipofectin" with DNA led to the formation of condensed structures as a result of interrelated lipid fusion and DNA collapse, as depicted in complex C of FIG. 4. The exposed surface of the condensed structure is considerably smaller than that of the extended DNA molecule.

2) DNA can form a complex with transferrin, as demonstrated by Sepharose 4B chromatography (See, FIG. 3D). Because transferrin is an ampholyte with an isoelectric point of 5.9, the small numbers of positively charged groups present in transferrin at pH 7.4 may be responsible for this interaction. Failure of transferrin to retard the mobility of DNA in agarose gel electrophoresis also supports the notion that the binding between DNA and transferrin is not very strong.

3) "Lipofectin" binds transferrin weakly (FIG. 3C) presumably through the negatively charged groups in transferrin at physiological pH. This interaction may not be strong enough to allow the "lipofectin"-transferrin complex to survive the conditions employed for sepharose 4B chromatography. However, it may be strong enough to permit transferrin to partially shield the positively charged groups of the "lipofectin" from binding to the DNA and at the same time enable the DNA to bind to "lipofectin" and transferrin. As a result, an efficient gene transfer complex is formed (FIG. 4, complex B).

4) The transfection reagents prepared by exposing "lipofectin" to DNA followed by a) transferrin, b) DNA and transferrin added simultaneously, or c) pre-formed DNA-transferrin complex, all have low transfection efficiency. These observations may be the result of the formation of ineffective gene transfer complexes as depicted in FIG. 4 (complex C). When all three components are present, "lipofectin"-DNA complex will be formed prior to the formation of other complexes, which leads to the formation of the inefficient gene transfer complexes.

In addition to its role in facilitating the entry of DNA to the cells, transferrin may play important roles in other steps of the gene expression process. For example, following the internalization of the "lipofectin"-DNA-transferrin-transferrin receptor complex, transferrin may facilitate the escape of DNA from the endosome. Because escaping from the endosome is a normal physiological process for transferrin and its receptor complex (Hueber (1987) supra; Aisen, (1994) supra) the entrapped DNA in the endosome may escape by following the transferrin and its receptor complex presumably via a bystander effect. Whether the enhancement of the transfection efficiency by transferrin is the result of this bystander effect or through other mechanisms remains to be elucidated. This mechanism may function as well with other cell receptor ligands, such as insulin.

It is believed that the scheme illustrated in FIG. 4 for "lipofectin" and transferrin is general and applicable to any receptor ligand including insulin, cholera toxin and the KNOB peptide and any mono- or polycationic liposome formulation and in particular also applies to "lipofectace" formulations and DC-cholesterol cationic liposomes with transferrin.

The high-efficiency gene transfer method of this invention particularly when monocationic lipids "lipofectin" or "lipofectace" are employed in combination with receptor ligands offers several advantages. The transfection reagent is easy to prepare, in several cases all of the ingredients are commercially available. Ligands prepared from one animal source may be employed for gene therapy in the same animal species, thus preventing host immune response to the vector. The host inflammatory and immune responses have been a major drawback for human gene therapy employing adenoviral vectors. (Ginsburg et al. (1991) supra; Trapnell et al. (1994) supra; Yang et al. (1994) supra; Yei et al. (1994) supra). Recent liposome toxicity studies in the lungs of humans show that liposome appears to cause minimal or no host inflammatory and immune response. (Thomas, D. A., Myers, M. A., Wichert, B., Schreier, H., and Gonzalez, R. J. (1991), "Acute effects of liposome aerosol inhalation on pulmonary function in healthy human volunteers," Chest. 99, 1268-1270) and several animal species (Stribling, R., Brunette, E., Liggitt, D., Gaensler, K., and Debs, R. (1992), "Aerosol gene delivery in vivo," Proc. Natl. Acad. Sci., USA 89, 11277-11281; Alton, E. W. F. W., Middleton, O. G., Caplen, N. J., Smith, S. N., Steel, D. M., Munkonge, F. M., Jeffery, P. K., Geddes, D. M., Hart, S. L., Williamson, R., Fasold, K. I., Miller, A. D., Dickinson, P., Stevenson, B. J., McLachlan, G., Dorin, J. R., and Porteous, D. J. (1993), "Non-invasive liposome-mediated gene delivery can correct the ion transport defect in cystic fibrosis mutant mice," Nature Genetics, 5, 135-142; Canonico, A. E., Plitman, J. D., Conary, J. T., Meyrick, B. O., and Brigham, K. L. (1994), "No lung toxicity after repeated aerosol or intravenous delivery of plasmid-cationic liposome complexes," J. Appl. Physiol., 77, 415-419; San, H., Yang, Z.-Y., Pompili, V. J., Jaffe, M. L., Plautz, G. E., Xu, L., Felgner, J. H., Wheeler, C. J., Felgner, P. L., Gao, X., Huang, L., Gordon, D., Nabel, G. J., and Nabel, E. G. (1993), "Safety and short-term toxicity of a novel cationic lipid formulation for human gene therapy," Hum. Gene Ther. 4, 781-788.) Utilization of human transferrin or other human ligands in combination with cationic liposomes may circumvent the host immune response while achieving high gene transfer efficiency in humans.

Transfection efficiency enhancement exhibited with transferrin differs among different cationic liposomes. As shown in Table 4, transferrin significantly enhances (10-fold or more enhancement of % blue cells or .beta.-Gal activity) transfection with "lipofectin" and "lipofectace" liposome formulations. Enhancement is also observed with DC-cholesterol liposomes, but no measurable enhancement is observed "lipofectamine", which contains the polycationic lipid DOSPA.

U.S. Pat. No. 4,897,355 (Eppstein et al.) issued in 1990 discloses cationic lipids related in structure to DOTMA. The cationic lipids and representative neutral lipids disclosed therein are useful in the cationic liposome formulations of this invention.

DDAB is the monocationic lipid dimethyl dioctadecylammonimum bromide. See U.S. Pat. No. 5,279,833 and WO 91/15501 (published Oct. 17, 1991 for DDAB and related cationic lipids). Related cationic species, for example, those in which the octadecyl groups are replaced with other higher alkyl groups (those alkyl groups having 8 or more carbon atoms); those in which the methyl groups are replaced with other lower alkyl groups (those alkyl groups having 1 to about 3 carbon atoms); or those in which the bromide anion is replaced with another anion are useful in the cationic liposome formulations of this invention.

Singhal, A. and Huang, L. (1994) "Direct Gene Transfer by Liposomes" J. Liposome Res.

4(1):289-299) describe cationic derivatives of cholesterol useful in preparation of cationic liposomes. Cholesterol derivatives in which a tertiary amino group is linked to a lipid anchor with an amide or carbamoyl bond and separated by a spacer of 3-6 atoms can be used to prepare liposomes with high transfection efficiency, increased shelf life and low toxicity. See also: Gao, X. and Huang, L. (1991) "A novel cationic liposome reagent for efficient transfection of mammalian cells" Biochem. Biophys. Res. Commun. 179:280-285; Farhood, H. Bottega, R. Epand, R. M. and Huang, L. (1992) "Effect of cationic cholesterol derivatives on gene transfer and protein kinase C activity" Biochem. Biophys. Acta 1111:239-246. DC-cholesterol is the derivitized cholesterol: 3β-[N-(N',N'-dimethylaminoethane)-carbamoyl]cholesterol. DC-cholesterol liposomes are prepared by combining the cationic lipid with a neutral lipid, for example DOPE.

"Lipofectamine" is a 3:1 liposome formulation of the polycationic lipid 2,3-dioleyloxy-N-[2(sperminecarboxamido)ethyl]-N,N-dimethyl-1-propanaminiu mtrifluoroacetate (DOSPA) and DOPE. See U.S. Pat. No. 5,334,761.

"Cellfecin" (Trademark) is a commercially available cationic liposome formulation which is a 1:1.5 (w/w) formulation of the polycationic lipid N,N',N",N"-tetrapalmitylspermine (TM-TPS) and DOPE. TM-TPS and related polycationic lipids useful in this invention are described in WO95/17373 (published Jun. 29, 1995).

U.S. patent application Ser. No. 08/195,866 filed Feb. 11, 1994 (Gebeyehu et al.) discloses other cationic lipids that are useful in the method of the present invention. Of particular interest are urethane derivatives disclosed therein.

U.S. Pat. No. 5,264,618 describes cationic lipids such as DOTAP (1,2-bis(oleoyloxy)-3-(trimethylammonio)propane. These lipids differ from DOTMA in that the oleoyl moieties are linked via ester rather than ether bonds to the propyl amine. A group of cationic lipids related in structure to DOTMA and DOTAP are those in which one of the methyl groups of the trimethylammonium group is replaced with a hydroxyethyl group. Compounds of this type are similar to the Rosenthal Inhibitor (RI) of phospholipase A (Rosenthal, A. F. and Geyer, R. P. (1960) J. Biol. Chem. 235:2202-2206) which has stearoyl esters linked to the propylamine core. The dioleoyl analogs of RI are commonly called DORI-ether or DORI-ester, depending on the linkage of the fatty acid moieties to the propylamine core. The hydroxyethyl group of such analogs can also be further functionalized for example by esterification. All of these cationic lipids structurally related DOTMA are useful in the cationic liposome formulations of this invention. Another cationic lipid useful in liposome formulations of this invention is 1,2-dimyristyloxypropanol-3,3-dimethylhydroxymethylammoniumbromide, which is often referred to as the acronym DMRIE.

As noted herein, this gene transfer method is generally applicable to any receptor ligand. Cotten and Wagner (1993) supra in a review of non-viral approaches to gene therapy provide, in Table 1 therein, a non-exhaustive list of species useful as receptor ligands for conjugate formation in receptor-mediated gene delivery. The specifics of the individual ligands is described in references cited therein. Other examples of receptor ligands for cell targeting are found in other references cited herein. Enhanced transfection of this invention has been demonstrated using transferrin, insulin and cholera toxin as receptor ligands. All of the ligands described in the Cotton and Wagner (1993) reference and other references cited herein can also function for enhanced transfection without covalent binding of the ligand as described in this invention. Therefore, this gene transfer protocol is applicable generally to ligands which can be internalized upon binding to cell surface receptors.

The specific examples provided herein show transient expression of DNA delivered to cells by receptor-facilitated liposome transfection. Failure to detect a population of the transfected cells which expressed .beta.-galactosidase suggests that despite the high-efficiency gene transfer obtained by using this transfection agent, integration of the plasmid DNA into the host genome is still a rare event. The receptor ligand facilitated gene transfer protocol of this invention can be employed with any known techniques for achieving integration of nucleic acid, e.g., DNA, into the host genomic DNA.

The method of this invention can be employed with any known techniques for enhancing expression of nucleic acid, e.g., DNA, introduced into the cell. For example, cointroduction of nuclear protein along with DNA in liposomes can result in enhanced express of the DNA. Kaneda, Y. Iwai, K. and Uchida, T. (1989) "Increased Expression of DNA Cointroduced with Nuclear Protein in Adult Rat Liver" Science 243:375-378; Kaneda, Y. Iwai, K. and Uchida, T. (1989) "Introduction and Expression of the Human Insulin

Gene in Adult Rat Liver" J. Biol. Chem. 264(21):12126-12129 Nuclear protein, such as non-histone chromosomal protein, high mobility group 1 (HMG-1), is believed to facilitate delivery of DNA to cell nuclei thus enhancing expression. The method of this invention can be employed to cointroduce DNA and nuclear protein to achieve further enhancement of expression.

Those of ordinary skill in the art will appreciate that certain details of the preparation of transfection agents of this invention and details of transfection protocols can be routinely varied to achieve optimal transfection efficiency and gene expression for a given receptor ligand, cationic lipid/neutral lipid liposome formulation, nucleic acid and target cell type. Such routine optimization is within the spirit and scope of their invention.

Those of ordinary skill in the art will also appreciate that all receptor ligands, cationic lipids, and neutral lipids other than those specifically exemplified herein can be employed in view of the descriptions herein in the compositions and methods of this invention.

The transferrin-cationic liposome protocol of this invention has been described for use in introduction of nucleic acids into cells. The transferrin-cationic liposome protocol of this invention can be readily adapted by reference to this disclosure and techniques and methods well known in the art to introduction of drugs and proteins as well as genes

into cells. Wagner et al. (1994) supra is a review of delivery of a variety of species using receptor-mediated endocytosis.

All references including journal articles, patents, and patent applications cited in this specification are incorporated in their entirety by reference herein. References cited provide inter also details of the structures of various cationic lipids and neutral lipids useful in preparation of cationic liposomes, methods of preparing liposomes and liposome formulations, sources of cell surface receptor ligands including methods for isolating or otherwise preparing such ligands, descriptions of cell growth conditions, details of the use of receptor ligands for targeted transfection and descriptions of in vitro and in vivo applications of transfection agents and methods.

The following examples illustrate the invention and are in no way intended to limit the scope of the invention.

THE EXAMPLES

Example 1

Transfection of HeLa Cells with "Lipofectin" and Transferrin

Methods:

Determination of Optimal Ratios of "Lipofectin" and DNA

Initial agarose gel electrophoresis was performed on mixtures of varying amounts (0.5-10 .mu.g) of "lipofectin" (Trademark) (GIBCO/BRL, Gaithersburg, Md.) at fixed amounts (1.5 .mu.g) of DNA (pCMVlacZ) (Clontech Lab, Inc., Palo Alto, Calif.) to determine the optimal ratio of "lipofectin" and DNA. The DNA in agarose gel was stained with ethidium bromide and visualized under a UV light. When complexed with "lipofectin", the DNA would not enter the gel. The same agarose gel electrophoresis procedure was employed to assess whether transferrin could retard DNA mobility by analyzing mixtures of 1.5 .mu.g DNA and 2-32 .mu.g transferrin.

Sepharose 4B Chromatography:

To assess whether transferrin was complexed with "lipofectin"-DNA complex, a transfection solution (500 .mu.L) which contained 32 .mu.g of [.sup.125 I] transferrin (7.times.10.sup.4 .mu.m) in 100 .mu.L HEPES-buffered saline (HBS) (20 mM HEPES, pH 7.4 and 100 mM NaCl), 3 .mu.g of "lipofectin", 1.5 .mu.g of pCMVlacZ, and 300 .mu.L of DMEM- H (GIBCO/BRL) was applied to a Sepharose 4B column (1.times.12 cm). The order of addition of transferrin, "lipofectin", and DNA varies according to the specific experimental protocol. The mixture was gently mixed and then incubated for 15 min after the addition of each reagent. Then, the column was developed with HBS, fractions of 0.5 mL collected, and radioactivity measured in a c-counter. "Lipofectin" was eluted at the

void volume (3 mL) and unbound [.sup.125 I] transferrin at 8.4-9.0 mL elution volume. DNA-"lipofectin"-transferrin and DNA-transferrin complexes appeared at the void volume.

Gene Transfer Protocol:

HeLa cells (American Type Culture, Rockville, Md.) which had been grown to confluence in a 48-well culture plate in DMEM-H medium containing 10% fetal bovine serum (FBS) were rinsed two times with serum-free DMEM-H. These cells were then exposed to 500 .mu.L of transfection solution prepared as described below. In 12.times.75 mm polystyrene tubes (Becton Dickinson, Lincoln Park, N.J.), the following reagents were sequentially added, gently mixed, and incubated for 15 min at room temperature after each addition: 100 .mu.L of HBS containing predetermined amounts of freshly prepared human transferrin (iron-saturated, heat inactivated, (Collaborative Biomedical Products, Becton Dickinson, Bedford, Mass.) (32 .mu.g for routine experiments), 3 .mu.L of "lipofectin" (1 mg/mL), and 100 .mu.L of HBS containing 1.5 .mu.g of pCMVlacZ. The mixture was transferred to each well which had been covered with 300 .mu.L of serum-free DMEM-H without antibiotics and then gently mixed. The plate was incubated for predetermined amounts of time (18-24 h for routine experiments) under water saturated environment and 5% CO.sub.2. The corresponding cultures exposed to "lipofectin"-DNA served as a control. The other two controls included DNA and DNA plus transferrin. The conditioned media were then replaced with 1.0 mL of DMEM-H containing 10% FBS and antibiotics (50 U/mL penicillin and 50 .mu.g/mL streptomycin). After culturing for 48 h, cells in three wells were fixed in 300 .mu.L of ice cold 2% paraformaldehyde--0.20% glutaraldehyde for 10 min following removal of the conditioned medium and washing twice with PBS. The cells were then exposed to 0.5 mL of 1.0 mg/mL 5-bromo-4-chloro-3-indolyl-.beta.-D-galactopyranoside (X-Gal) (GIBCO/BRL) (Yin and Cheng (1994) "Lectin conjugated-directed gene transfer to airway epithelial cells," Biochem. Biophys. Res. Commun. 205:826-833), at 37.degree. C. for two days after the fixatives had been removed and rinsed twice with PBS. The percentage of blue cells was measured by counting the number of blue cells out of a total of 2,500-3,000 cells in 20-30 randomly chosen fields under a phase-contrast microscope. Cells in three other wells were rinsed three times with PBS, dissociated by trypsinization, rinsed three times with PBS, and then lysed by three cycles of freezing and thawing. These lysates were measured for .beta.-galactosidase activity as light units (Beale et al. (1992) "A rapid and simple chemiluminescent assay for Escherichia coli .beta.-galactosidase," Biotechniques 120:320-324) on a Lummat LB9501 spectrofluorimeter (EG&G Berthold, Nashua, N.H.) and measured for protein (Bradford Reagent, Bio-Rad, Melville, N.Y.) using bovine serum albumin (BSA) as the standard. The data were expressed as light units per .mu.g protein.

Transfer of .sup.35 S-pCMVlacZ to HeLa Cells:

.sup.35 S-labelled pCMVlacZ was prepared by random priming (Boehringer-Mannheim, Indianapolis, Ind.) after linearization by EcoRI digestion. Each of the four transfection solutions, DNA, DNA+transferrin (32 .mu.g), DNA+"lipofectin" (3 .mu.g), and DNA+"lipofectin"+transferrin, contained 1.5 .mu.g of .sup.35 S-labelled pCMVlacZ (1.4.times.10.sup.4 cpm). At 0, 0.5, 1, 3, 6, and 24 h after transfection, media were removed. Then, the cells were washed once with 1 mL HES, recovered by trypsinization, and washed twice with 1.5 mL HES. The pelleted cells were suspended in 60 .mu.L of distilled water and ruptured by freezing and thawing three times. Aliquots were measured for radioactivity and protein. The data were expressed as cpm/mg protein.

Other Methods:

pCMVlacZ was purified from Escherichia coli, which harbors this plasmid DNA. using a QiaGen plasmid isolation kit (Chatsworth, Calif.). [.sup.125 I] Transferrin was prepared by a chloramine-T method as described below. 5 .mu.L of 100 mCi/mL Na.sup.125 I (Amersham, Arlington Hts., Ill.) and 5 .mu.L of 2 mg/mL chloramine T (Eastman Kodak Company, Rochester, N.Y.) were added to 100 .mu.L HBS containing 100 .mu.g transferrin. After incubation for 20-30 sec, 5 .mu.L of sodium metabisulfite (Sigma, St. Louis, Mo.) was added to stop the reaction. The [.sup.125 I] transferrin was isolated on sephadex G-25 (0.5.times.4 cm).

Statistical Analysis:

Statistical analysis was performed by a two-tailed, unpaired t-test program (GaphPAD InPlot Software, San Diego, Calif.).

RESULTS

Gene Transfer Efficiency as a Function of Transfection Times:

Exposure of HeLa cells to the transfection solution composed of transferrin, "lipofectin," and pCMVlacZ for various periods of time from 0.5 to 24 hours resulted in increased expression of .beta.-galactosidase as assessed by both .beta.-galactosidase activity and percentage of blue cells (Table 1). A 2.5-fold increase of .beta.-galactosidase activity in the cells transfected with the reagent prepared from transferrin, "lipofectin", and DNA over that of the control (p=0.0005) was observed after 0.5 h exposure even though the % blue cells did not differ between these two groups. Exposure for 1 h gave rise to 13-fold increase in .beta.-galactosidase activity and 30% blue cells. After 5 h exposure, essentially all the cells were transfected. Similarly, .beta.-galactosidase activity increased steadily for up to 5 hours and began to level off afterwards. For routine gene transfer experiments, overnight transfection was chosen in order to maximize the expression of .beta.-galactosidase activity. In other controls which employed DNA or DNA plus transferrin, <0.01% of the cells were transfected.

Transfection Efficiency as a Function of Transferrin Amounts:

The transfection efficiency obtained with the mixture of transferrin, "lipofectin", and DNA was dependent on the amounts of transferrin employed. As shown in Table 2, the percentage of cells transfected and the .beta.-galactosidase activities expressed in the transfected cells were increased when .ltoreq..mu.g of transferrin were used. In addition, when the amounts of transferrin used reached 8 .mu.g and above, at least 97% of the HeLa cells were transfected. However, maximal expression of .beta.-galactosidase activity was not obtained until 16 .mu.g of transferrin was used.

Expression of Transfected Reporter Gene as a Function of Passage Numbers:

The high transfection efficiency in HeLa cells mediated by "lipofectin" plus transferrin afforded an excellent opportunity to examine the question for how many passages would the expression of the delivered reporter gene be maintained. As shown in FIG. 1 and Table 3, the percentage of transfected cells that were stained blue decreased progressively from 70% after one passage to <0.01% after 6 passages. Progressive decrease of .beta.-galactosidase activity as a function of passage number was also observed.

Characterization of the Transfection Agent:

Agarose gel electrophoresis of the mixtures that contained fixed amount of DNA (1.5 .mu.g) and varying amounts of "lipofectin" (0.5-10 .mu.g) showed that all the DNA was complexed with "lipofectin" when "lipofectin" was at least two-to-three times the DNA amounts (w/w). This result formed the basis for the experimental protocol of employing 3 .mu.g of "lipofectin" and 1.5 .mu.g of DNA for each well of the 48-well plate.

To assess whether the observed increased expression of .beta.-galactosidase was the result of increased transfer of DNA, a gene transfer experiment employing [.sup.35 S]DNA was performed. As shown in FIG. 2, in the absence of "lipofectin", transferrin did not increase DNA transfer over that with DNA alone. The maximal amount of DNA transferred by the "lipofectin"-DNA complex was at least two times that by DNA only. Incubation of transferrin with "lipofectin" prior to the addition of DNA doubled the amount of DNA delivered by "lipofectin"-DNA complex. By 24 h, the amount of DNA found in the cells transfected with the reagent prepared by sequential addition of transferrin, "lipofectin", and DNA decreased by 40%.

To assess the interactions among the different components in the transfection solution, Sepharose 4B column chromatography was performed. Analysis of the transfection solution prepared by sequential addition of 32 .mu.g [.sup.125 I]transferrin, 3 .mu.g "lipofectin", and 1.5 .mu.g DNA showed that 6.8% of total radioactivity was eluted in the void volume (FIG. 3A). No radioactivity was detected in the void volume when the solution was prepared in the order of DNA, "lipofectin", followed by [.sup.125 I]transferrin (FIG. 3B). The transfection efficiency (3.2%) employing the reagent prepared the same way was not different from the control value (3.9%) obtained by transfection with "lipofectin" plus DNA. Only a small amount (0.2%) of radioactivity was detected in the void volume when the mixture of [.sup.125 I]transferrin and "lipofectin" was analyzed (FIG. 3C). In addition, when [.sup.125 I]transferrin was mixed with DNA, 6.8% of the radioactivity was also found in the void volume (FIG. 3D). The transfection efficiency (2.8%) employing transferrin plus DNA was the same as the

control (data not shown). In addition, addition of 2-32 .mu.g transferrin to 1.5 .mu.g DNA did not decrease the mobility and the band intensity of the DNA on agarose gel electrophoresis.

Variation of Transferrin-facilitated pCMVlacZ Transfection Efficiency with Different Cationic Liposomes

The transfection efficiency of transferrin-facilitated gene transfer in HeLa cells varied with different cationic liposomes. As shown in Table 4, "lipofectin" was the most effective liposome among the four different cationic liposomes examined. This was followed by "lipofectace" and then DC-cholesterol. "Lipofectamine" exhibited no enhancement in the results listed in Table 4. It may be, however, that the experiment with "lipofectamine", the results of which are listed in Table 4, does not accurately reflect the usefulness of "lipofectamine" as a cationic liposome component in methods of this invention. "Lipofectamine" only is generally found to be a reasonably efficient transfection agent into HeLa cells. Transfection efficiences of 40% of DNA are routinely observed with "lipofectamine" alone. See Hawley-Nelson, P. et al. (1993) FOCUS 15:73. The percentage of blue cells observed in the experiment of Dable 4 for "lipofectamine" alone appears to be significantly lower than would normally be expected. This suggests some problems with the transfection experiment.

EXAMPLE 2

Correction of the Chloride Transport Abnormality of CFT1 Cells by Transferrin-facilitated Gene Transfer Mediated by Cationic Liposomes

CFT1 cells grown to confluence in 48-well plates were exposed for 24 h to a transfection solution that contained "lipofectin", DNA, and transferrin.

The transfection solution was prepared as in Example 1. Specifically, transferrin was added to "lipofectin" and the mixture was incubated at room temperature for about 15 min. Thereafter, the DNA was added and the mixture was again incubated for about 15 min at room temperature.

The transfected cells were further cultured for 2-3 days before being fixed in glutaraldehyde and then developed with X-gal for 2-3 days. The transfection efficiency (% blue cells) was dependent on transferrin concentration (.mu.g/0.5 mL transfection solution); 0 .mu.g, 0.1%; 1 .mu.g, 0.46%; 2 .mu.g, 5.5%; 4 .mu.g, 7%; 8 .mu.g, 8%; 16 .mu.g, 10%; and 32 .mu.g, 11%. The .beta.-galactosidase activities measured by the Lumigal method also followed the same trend (see FIG. 5). Using the protocol that yielded the highest transfection efficiency to deliver pCMVlacZ to CFT1 cells grown in 35 mm dishes, a 33% transfection efficiency was obtained. CFT1 cells transfected with pCMVCFTR using this protocol exhibited a greater rate of .sup.36 Cl.sup.- effux than CFT1 cells transfected by "lipofectin" without transferrin or CFT1 cells without transfection (see FIG. 6). These results indicate that the transferrin-facilitated CFTR gene transfer mediated by liposome can correct the chloride transport abnormality of CF airway epithelial cells. Significant correction of the chloride transport abnormality was observed when insulin or cholera toxin were employed for transfection with "lipofectin". (See FIG. 7.)

METHODS

Transfection Protocol and Measurement of Transfection Efficiency:

The confluent CFT1 cells grown in a 48-well plate were exposed overnight to a transfection solution (0.5 mL), which contained 3 .mu.g "lipofectin", 1.5 .mu.g DNA, and 1-32 .mu.g transferrin. The transfected cells were then cultured in F-12 medium supplemented with 7 different hormones and growth factors for 2-3 days. Then, cells in 2 wells were fixed in 4% paraformaldehyde at 4.degree. C. for 10 min and exposed to X-gal for 2-3 days. The transfection efficiency was determined by the number of blue cells per 100 cells counted under a phase contrast microscope. Cells in 3 other wells were recovered by trypsinization and their homogenates were measured for .beta.-galactosidase activities by the Lumigal method (Beale et al. (1992) supra. The data were expressed as light units/.mu.g protein. See FIG. 5.

.sup.36 Cl.sup.31 Efflux Assay:

CFT1 cells were grown to confluence in a 6-well plate. Cells in one well were treated with "lipofectin" and pCMVCFTR plus transferrin and cells in 2 other wells were treated

with "lipofectin" and pCMVlacZ plus transferrin as described above. The first two wells were measured for .sup.36 Cl.sup.- efflux and the third well was fixed, treated with X-gal, and measured for % blue cells. The first 2 wells were loaded with 5-10 .mu.Ci of .sup.31 Cl.sup.-, which was followed by a quick rinse with Cl.sup.- free medium. Then, 1 mL aliquots of isotope-free and Cl.sup.- free medium plus 0.1 mM amiloride was added and removed at 1 min intervals for up to 10 min. At the 3 min time point, 10 .mu.M forskolin was added to assess cAMP-mediated Cl.sup.- permeability. At the end of 10 min the cell-associated isotope

was measured in 0.1% SDS extract of the cells. The data were analyzed by plotting the percentage of .sup.36 Cl.sup.- remaining as a function of time. See FIG. 6.

Transfection Employing .sup.36 Cl.sup.- Insulin or Cholera Toxin

Employing 6 mL of transfection solution which contains 36 .mu.g "lipofectin" plus 380 .mu.g transferrin, 0.2 .mu.g insulin, or 160 .mu.g cholera toxin to deliver 18 .mu.g pCMVCFTR to confluent CFT1 cells in each well of 6.times.35 mm plate, significant correction of the chloride transport abnormality was observed (FIG. 7). In a contemporaneous experiment, employing identical protocol to deliver 18 .mu.g pCMVlacZ to CFT1 cells resulted in significant numbers of cells stained blue, i.e. "lipofectin", 0.5%; "lipofectin"+transferrin, 33%; "lipofectin"+insulin, 13%; and "lipofectin"+cholera toxin, 31%. These results demonstrate that it is feasible to restore the cAMP-dependent chloride conductance in CF airway epithelial cells using a wild type CFTR cDNA and the transfection vectors composed of receptors and a cationic liposome.

EXAMPLE 3

Liposome Mediated Transfection Facilitated by Insulin and Cholera Toxin.

Transfection solutions were prepared as described in Example 1 wherein the receptor ligand was insulin or cholera toxin. FIGS. 8a and 8b show the insulin concentration-dependent transfection efficiency in HeLa cells and CFT1 cells, respectively. FIGS. 9a and 9b show the cholera toxin concentration-dependent transfection efficiency in HeLa cells and CFT1 cells, respectively. Transfection protocols were performed as in Example 1 for HeLa cells and as in Example 2 for CFT1 cells.

Transfection efficiency did not increase when CFT1 cells were transfected with a solution containing a mixture of the three different ligands (transferrin, insulin and cholera toxin). This suggests interference of gene transfection of one receptor ligand by another.

EXAMPLE 4

Components of Human Serum that Facilitate Gene Transfer Mediated by "Lipofectin"

The transferrin concentration in human serum is around 2.2-3.7 mg/mL. High transfection efficiency was expected by using 5-10 .mu.L of serum in place of the transferrin receptor ligand. Only marginal transfection efficiency was observed, however, suggesting interference of transferrin-facilitated gene transfection by other human serum components. Maximal gene transfer efficiency was obtained when 0.01 .mu.L of serum was used in place of transferrin in the protocol of Example 1 (FIG. 10).

To characterize different serum components which produce high transfection efficiency, a human serum sample was fractionated by protein A-column into IgG-depleted (protein A-:run-through) and IgG-enriched (protein A+: bound and recovered by low pH) fractions. The same serum sample was also fractionated by concanavalin A-column into run-through or Con A-fraction, which contains proteins and mannose-free glycoproteins, and the fraction recovered from Con A, which contains mannose-bound (Con A+) glycoproteins. The transfection efficiencies of protein A fractions are shown in FIG. 11a and Con A fractions in FIG. 11b.

The component(s) responsible for the high transfection efficiency found with 0.01 .mu.l serum appear to be present in protein A- and Con A- fractions but not in the other two fractions. These results indicate that some IgG's and mannose-containing glycoproteins can efficiently facilitate "lipofectin"-mediated gene transfer. Since low transfection efficiency was found using an amount of the protein A- fraction which contained 11-37 .mu.g of transferrin (this concentration of transferrin should yield >80% transfection

efficiency), the serum components which interfere with the transferrin-facilitated gene transfer are present substantially in protein A- but not in Con A+ fraction.

Serum samples were also fractionated according to size using centrifugation filters with MW cut-off of 100 and 30 Kd. As shown in FIG. 12, the component(s) responsible for the high transfection efficiency obtained with 0.01 .mu.l serum appear to have MW greater than 100 Kd. Both the 30-100 Kd and <30 Kd fractions contain compounds which yield high transfection efficiency. Transferrin, which has a MW of 89 Kd, is at least in part responsible for the high-efficiency gene transfer exhibited by the 30-100 Kd fraction. In addition, compounds which interfere with the Tf-facilitated gene transfer were present in the >100 Kd and also in <30 Kd fractions.

TABLE 1

Transferrin and Lipofection-Directed Gene Transfer to Hela Cells: Gene Transfer Efficiency as a Function of Transfection Times (0.5-24 h).

A detailed experimental protocol is described in the Examples. At the end of a 48-hour culture period after transfection with the agent prepared from 16 .mu.g transferrin, 3.mu.g "lipofectin", and 1.5 .mu.g pCMVlacZ, lysates of the cells in 3 wells were measured separately for .beta.-galactosidase activity by the Lumigal method (Beale et al., 1992). .beta.-Galactosidase activity was expressed as light units (mean .+-.S.E.M.) per tg protein. Hela cells in 3 other wells were stained with X-gal and % of blue cells measured.

Time (h)	Transfection with Transferrin-Lipofectin .beta.-Galactosidase Activity (Light Units/ .mu.g protein)	Blue Cells (%)	Transfection with Lipofectin .beta.-Galactosidase Activity (Light Units/ .mu.g protein)	Blue Cells (%) *
0.5	2157 .+-. 144	0.9	616 .+-. 32	0.6
1.0	4976 .+-. 357	30.4	353 .+-. 17	1.2
3.0	10113 .+-. 402	90.0	600 .+-. 210	3.1
5.0	25011 .+-. 1979	97.1	180 .+-. 71	3.3
6.0	26350 .+-. 3535	99.0	414 .+-. 17	3.8
24.0	33769 .+-. 5733	100.0	522 .+-. 138	4.0

* < 0.01% blue cells were found when the cells were treated with 1.5 .mu. DNA or DNA plus 32 .mu.g of transferrin.

TABLE 2

Transferrin and Lipofectin-Directed Gene to Hela Cells: Gene Transfer Efficiency as a

Function the Amounts (1-32 .mu.g) of Transferrin.

Transfection solution (500 .mu.L) contained 3 .mu.g of "lipofectin", 1.5 .mu.g of pCMVlacZ, and varying amounts of transferring indicated. The transfection time was 18 hours. Detailed experimental protocol is described in the Examples and Table 1.

Transferrin (.mu.g)	.beta.-Galactosidase Activity (Light Units/.mu.g Protein)	Blue Cells (%)
0	1923 .+-. 90	3.1
1	1600 .+-. 95	4.3
2	2333 .+-. 398	6.5
3	8210 .+-. 2770	36.0
4	19163 .+-. 6283	86.0
8	86143 .+-. 12261	97.0
16	235920 .+-. 41472	98.0
32	187393 .+-. 10635	98.2*

*Transfection efficiency, which varied from experiment to experiment, was between 55 and 100%. This variation may be attributable to different batches of reagents.

TABLE 3

Expression of .beta.-Galactosidase in Hela Cells 100% Transfected with Transferrin, Lipofectin and pCMVlacZ (F0) and in the Transfected Cells of Six Consecutive Passages (F1 to F6).

The transfection solution contained 3.0 .mu.g of lipofectin, 16 .mu.g of transferrin, and 1.5 .mu.g of DNA. Cells transfected with "lipofectin" plus DNA, but without transferrin served as the control. Transfection time was 18 h. The experimental protocol is described in the Examples and FIG. 1.

HeLa Cells (Passages)	Transfection with Transferrin-Lipofectin .beta.-Galactosidase Activity (Light Units/.mu.g protein)	Transfection with Transferrin-Lipofectin Blue Cells (%)	Transfection with Lipofectin .beta.-Galactosidase Activity (Light Units/.mu.g protein)	Transfection with Lipofectin Blue Cells (%)
F0	87546 .+-. 15140	99.8	1204 .+-. 94	3.0
F1	15233 .+-. 996	69.6	460 .+-. 31	

				0.54
F2	5981 .+-. 950	12.3	431 .+-. 37	0.23
F3	3325 .+-. 507	6.3	362 .+-. 59	0.02
F4	1450 .+-. 217	1.6	120 .+-. 43	<0.01
F5	550 .+-. 128	0.4	322 .+-. 16	<0.01
F6	240 .+-. 27	<0.01	129 .+-. 61	<0.01

TABLE 4

Transferrin-Enhanced pCMVlacZ Transfection Efficiency in HeLa Cells Using Different Cationic Liposome

A detailed experimental protocol is described in Table 1 and FIG. 1. The amounts of transferrin and the four different cationic liposomes used were: transferrin, 32 .mu.g; "lipofectin", 3 .mu.g; "lipofectace" 4.2 .mu.g; "lipofectamine" 4 .mu.g; and DC-cholesterol, 4 nmoles.

Liposome	Transfection with Transferrin-Liposome .beta.-Galactosidase Activity (Light Units/ .mu.g protein)	Blue Cells (%)	Transfection with Liposome .beta.-Galactosidase Activity (Light Units/ .mu.g protein)	Blue Cells (%)
"Lipofectin"	20,047 .+-. 3752	55	1,387 .+-. 61	5.5
"Lipofectace"	6,428 .+-. 829	35	273 .+-. 59	2.7
"Lipofectamine"	1,133 .+-. 49	7.1	796 .+-. 125	7.9
DC-Cholesterol	884 .+-. 107	10	379 .+-. 30	4.2

CLAIMS:

What is claimed is:

1. A method for intracellular delivery of a polynucleotide comprising:

(a) first combining a non-viral receptor ligand and a cationic lipid to form a mixture, so that said ligand and lipid become associated although not covalently bound; and thereafter

(b) adding to said mixture a polynucleotide, so that said polynucleotide becomes associated with said lipid to form a molecular mixture; and

(c) introducing said molecular mixture to a cell, wherein said molecular mixture enhances the delivery of said polynucleotide to said cell.

2. The method of claim 1 further comprising the step of:

incubating said mixture prior to addition of the polynucleotide.

3. The method of claim 1 further comprising the step of:

incubating said molecular mixture prior to introduction of said molecular mixture to said cell.

4. The method of claim 1 wherein said cationic lipid is in a liposome formulation with a neutral lipid to form a cationic liposome formulation.

5. The method of claim 4 wherein said neutral lipid is dioleoyl phosphatidylethanolamine.

6. The method of claim 4 wherein said liposome formulation is a formulation of N-[1-(2,3-dioleyloxy)propyl]-N,N,N-trimethyl and dioleoyl phosphatidylethanolamine.

7. The method of claim 4 wherein said liposome formulation is a formulation of 2,3-dioleyloxy-N-[2(spermine carboxamido)ethyl]-N,N-dimethyl-1-propanamintrumtrifluoroacetate and dioleoyl phosphalidylethanolamine.

8. The method of claim 4 wherein said formulation has 2,3-dioleyloxy-N-[2(spermine carboxamido)ethyl]-N,N-dimethyl-1-propanamintrumtrifluoroacetate and dioleoyl phosphalidylethanolamine.

9. The method of claim 4 wherein said liposome formulation is a formulation of N,N',N",N"'-tetrapalmetylspermine and dioleoyl phosphatidylethanolamine.

10. The method of claim 1 wherein said cationic lipid is selected from the group consisting of:

N-[1-(2,3-dioleyloxy)propyl]-N,N,N-trimethyl, dimethyl dioctadecylammonimum bromide; DOSPA; DL-1,2-dioleoyl-3-dimethyl-aminopropyl-B-hydroxyethylammonium; DL-1,2-O-dioleyl-3-dimethylaminopropyl-B-hydroxyethylammonium; DL-1-O-oleyl-2-oleoyl-3-dimethylaminopropyl-B-hydroxyethylammonium; DMRIE; DL-1,2-dioleoyl-3-propyl-N,N,N-trimethylammonium; and TM-TPS.

11. The method of claim 1 wherein said receptor ligand is selected from the group consisting of:

transferrin, insulin, and cholera toxin.

12. The method of claim 1 wherein said polynucleotide is a deoxyribonucleic acid.

13. The method of claim 1 wherein said polynucleotide is a ribonucleic acid.

14. The method of claim 1 wherein said polynucleotide encodes a gene product.

15. The method of claim 1 wherein said cell is in vitro.

16. The method of claim 1 wherein said cell is in vivo.

17. The method of claim 1 wherein said cell is an animal cell.

18. A method for intracellular delivery of a naked nucleotide sequence without the use of a vector comprising:

(a) first combining a human protein receptor ligand and a cationic lipid to form a mixture, so that said ligand and lipid become associated although not covalently bound; and thereafter

(b) adding to said mixture a naked nucleotide molecule to be delivered to a cell so that said nucleotide molecule becomes associated with said lipid to form a nucleotide mixture, and thereafter

(c) introducing said nucleotide mixture to a cell, wherein said nucleotide mixture enhances the delivery of said molecule to said cell.

19. A method for intracellular delivery of a polynucleotide comprising:

(a) first combining a transferrin receptor ligand and a cationic lipid to form a mixture, so that said ligand and lipid become associated although not covalently bound; and thereafter

(b) adding to said mixture a polynucleotide to be delivered to a cell to form a molecular mixture; and thereafter

(c) introducing said molecular mixture to a cell, wherein said molecular mixture enhances the delivery of said polynucleotide to said cell.

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L13: Entry 28 of 38 File: USPT May 2, 2000

US-PAT-NO: 6056973
DOCUMENT-IDENTIFIER: US 6056973 A

TITLE: Therapeutic liposome composition and method of preparation

DATE-ISSUED: May 2, 2000

INVENTOR-INFORMATION:

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Uster; Paul	Tracy	CA		
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US-CL-CURRENT: 424/450; 436/829

CLAIMS:

It is claimed:

1. A kit containing reagents for use in preparing a therapeutic liposome composition sensitized to a target cell, comprising

a liposomal composition composed of pre-formed liposomes having an entrapped therapeutic agent; and

a plurality of conjugates, each conjugate composed of (i) a lipid having a polar head group and a hydrophobic tail, (ii) a hydrophilic polymer having a proximal end and a distal end, said polymer attached at its proximal end to the head group of the lipid, and (iii) a targeting ligand attached to the distal end of the polymer;

wherein a therapeutic, target-cell sensitized liposome composition is formed by incubating the liposomal composition with a selected conjugate.

2. The kit of claim 1, wherein the targeting ligand is an antibody or an antibody fragment.

3. The kit of claim 2, wherein the antibody or antibody fragment is a humanized murine antibody.

4. The kit of claim 2, wherein the targeting ligand specifically binds to an extracellular domain of a growth factor receptor.

5. The kit of claim 4, wherein the receptors are selected from the group consisting of c-erbB-2 protein product of the HER2/neu oncogene, epidermal growth factor receptor, basic fibroblast growth factor receptor, and vascular endothelial growth factor receptor.

6. The kit of claim 2, wherein the targeting ligand binds a receptor selected from the group consisting of E-selectin receptor, L-selectin receptor, P-selectin receptor, folate receptor, CD4 receptor, CD19 receptor, .alpha..beta. integrin receptors and chemokine receptors.

7. The kit of claim 1, wherein the targeting ligand is selected from the group consisting of folic acid, pyridoxal phosphate, vitamin B12, sialyl Lewis.sup.x,

transferrin, epidermal growth factor, basic fibroblast growth factor, vascular endothelial growth factor, VCAM-1, ICAM-1, PECAM-1, RGD peptides and NGR peptides.

8. The kit of claim 1, wherein the targeting ligand binds a receptor on a malignant B-cell or T-cell, said receptor selected from the group consisting of CD19, CD20, CD22, CD4, CD7 and CD8.

9. The kit of claim 1, wherein the hydrophilic polymer is selected from the group consisting of polyvinylpyrrolidone, polyvinylmethylether, polymethyloxazoline, polyethyloxazoline, polyhydroxypropyloxazoline, polyhydroxypropylmethacrylamide, polymethacrylamide, polydimethylacrylamide, polyhydroxypropylmethacrylate,

polyhydroxyethylacrylate, hydroxymethylcellulose, hydroxyethylcellulose, polyethyleneglycol, polyaspartamide and hydrophilic peptide sequences.

10. The kit of claim 1, wherein the hydrophilic polymer is polyethylene glycol.

11. The kit of claim 10, wherein the polyethylene glycol has a molecular weight between 500-5,000 daltons.

12. The kit of claim 1, wherein the entrapped therapeutic agent is a cytotoxic drug.

13. The kit of claim 12, wherein the cytotoxic drug is an anthracycline antibiotic selected from the group consisting of doxorubicin, daunorubicin, epirubicin and idarubicin and analogs thereof.

14. The kit of claim 12, wherein the cytotoxic agent is a platinum compound selected from cisplatin, carboplatin, ormaplatin, oxaliplatin, zeniplatin, enloplatin, lobaplatin, spiroplatin, ((-)-(R)-2-aminomethylpyrrolidine (1,1-cyclobutane dicarboxylato)platinum), (SP-4-3(R)-1,1-cyclobutane-dicarboxylato(2-)-(2-methyl-1,4-butanediamine-N ,N')platinum), nedaplatin and (bis-acetato-ammine-dichloro-cyclohexylamine-platinum(IV)).

15. The kit of claim 12, wherein the cytotoxic agent is a topoisomerase 1 inhibitor selected from the group consisting of topotecan, irinotecan, (7-(4-methylpiperazino-methylene)-10,11-ethylenedioxy-20(S)-camptothecin), 7-(2-(N-isopropylamino)ethyl)-(20S)-camptothecin, 9-aminocamptothecin and 9-nitrocamptothecin.

16. The kit of claim 12, wherein the cytotoxic agent is a vinca alkaloid selected from the group consisting of vincristine, vinblastine, vinleurosine; vinrodisine, vinorelbine and vindesine.

17. The kit of claim 1, wherein the entrapped agent is a nucleic acid.

18. The kit of claim 17, wherein the nucleic acid is an antisense oligonucleotide or ribozyme.

19. The kit of claim 17, wherein the nucleic acid is a plasmid containing a therapeutic gene which when internalized by the target cells achieves expression of the therapeutic gene to produce a therapeutic gene product.

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L5: Entry 14 of 23 File: USPT Jul 7, 1998

DOCUMENT-IDENTIFIER: US 5777153 A
TITLE: Cationic lipids

Brief Summary Paragraph Right (29):
The invention lipid-polyanion complexes are believed to form micelles or liposomes of about 40 to 600 nm in diameter. Light scattering experiments using a 1:1 molar ratio of 34 cationic lipid and DOPE colipid (prepared by sonication as described in Example 5 below) showed two peaks corresponding to particles of about 66 nm and about 260 nm in diameter, with lesser amounts of particles above, below and between these sizes.

Brief Summary Paragraph Right (30):
The invention cationic lipids 30 or 34 and DOPE colipid (1:1) were prepared by sonication and then filtered using 200, 100 or 50 nm filters to obtain particles less than about 200 nm in diameter, less than about 100 nm and less than about 50 nm. Transfection efficiency using 200 nm filtered or 100 nm filtered preparations were the most efficient (and equally efficient) with regard to both the proportion of cells transfected and the amount of nucleic acid delivered per cell. The 50 nm filtered preparations were about 40-50% as efficient as the preparations containing larger particles. The size of the particles or micelles in a given preparation will vary depending on the preparation method. Sonicating cationic lipid-colipid mixtures will provide smaller micelles and vortexing will provide larger micelles (Felgner J. Biol. Chem. (1994) 269:2550-2561). Such micelles are believed to transfer the polyanion into the cytoplasm of a eukaryotic or prokaryotic cell by pinocytosis, endocytosis and/or by direct fusion with the plasma membrane.

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L13: Entry 15 of 38 File: USPT Oct 16, 2001

DOCUMENT-IDENTIFIER: US 6303378 B1
TITLE: Methods for preparing polynucleotide transfection complexes

Brief Summary Paragraph Right (3):
Generally, the various methods used to introduce nucleic acids into cells have as a goal the efficient uptake and expression of foreign genes. In particular, the delivery of exogenous nucleic acids in humans and/or various commercially important animals will ultimately permit the prevention, amelioration and cure of many important diseases and the development of animals with commercially important characteristics. The exogenous genetic material, either DNA or RNA, may provide a functional gene which, when expressed, produces a protein lacking in the cell or produced in insufficient amounts, or may provide an antisense DNA or RNA or ribozyme to interfere with a cellular function in, e.g., a virus-infected cell or a cancer cell, thereby providing an effective therapeutic for a disease state.

Detailed Description Paragraph Right (9):
"Polycation" refers to any molecular entity having multiple positive charges, which, when combined with nucleic acid, interacts by ionic interactions with the nucleic acid. "Polycationic carrier" refers to a polycation which, when combined with a polynucleotide, forms a complex suitable for transfecting eukaryotic cells. For example, cationic lipids have been shown to be efficient polycationic carriers for nucleic acid delivery to cells. Typically, cationic lipid carriers are in the form of liposomes having both cationic and non-cationic lipid (usually neutral lipid) components. Thus, a "lipid carrier" or "cationic lipid carrier" refers to a lipid composition of one or more cationic lipids and, optionally, one or more non-cationic lipids for delivering agents to cells. The lipid carrier may be in any physical form including, e.g., liposomes, micelles, interleaved bilayers, etc.

Detailed Description Paragraph Right (11):
Lipid carriers usually contain a cationic lipid and a neutral lipid, usually in approximately equimolar amounts. The neutral lipid is helpful in maintaining a stable lipid bilayer in liposomes, and can significantly affect transfection efficiency. The liposomes may have a single lipid bilayer (unilamellar) or more than one bilayer (multilamellar). They are generally categorized according to size, where those having diameters up to about 50 to 80 nm are termed "small" and those greater than about 80 to 1000 nm, or larger, are termed "large." Thus liposomes are typically referred to as large unilamellar vesicles (LUVs), multilamellar vesicles (MLVs) or small unilamellar vesicles (SUVs). Methods of producing cationic liposomes are known in the art. See, e.g., Liposome Technology (CFC Press, NY 1984); Liposomes by Ortro (Marcel Schher, 1987); Methods Biochem Anal. 33:337462 (1988).

Detailed Description Paragraph Right (12):
Cationic lipids of interest include, for example, imidazolinium derivatives (WO 95/14380), guanidine derivatives (WO 95/14381), phosphatidyl choline derivatives (WO 95/35301), and piperazine derivatives (WO 95/14651). Examples of cationic lipids that may be used in the present invention include DOTIM (also called BODAI) (Solodin et al., (1995) Biochem. 34: 13537-13544), DDAB (Rose et al., (1991) BioTechniques 10(4):520-525), DOTMA (U.S. Patent No. 5,550,289), DOTAP (Eibl and Wooley (1979) Biophys. Chem. 10:261-271), DMRIE (Felgner et al., (1994) J. Biol. Chem. 269(4): 2550-2561), EDMPC (commercially available from Avanti Polar Lipids, Alabaster, Alabama), DCChol (Gau and Huang (1991) Biochem. Biophys. Res. Comm. 179:280-285), DOGS (Behr et al., (1989) Proc. Natl. Acad. Sci. USA, 86:6982-6986), MBOP (also called MeBOP) (WO 95/14651), and those described in WO 97/00241. Particularly preferred are EDMPC for aerosolized delivery to airway epithelial cells, and DOTIM, DOTAP or MBOP for intravenous delivery to vascular endothelial cells of various organs, particularly the

lung. In addition, cationic lipid carriers having more than one cationic lipid species may be used to produce complexes according to the method of the present invention.

Detailed Description Paragraph Right (14):
Additional polycationic carriers include positively charged peptides and proteins, both naturally occurring and synthetic, as well as polyamines, carbohydrates or synthetic polycationic polymers. Examples include polylysine, polyarginine, protamnine, polybrene, histone, cationic dendrimer, and synthetic polypeptides based on viral peptides, e.g., having cell binding, endosomal release or nuclear localizing functions, etc. For certain applications, polycationic carriers may include cationic lipid as well as peptide moieties. See, e.g., WO 96/22765.

Detailed Description Paragraph Right (16):
"Transfection complex" or "polynucleotide transfection complex" refers to a combination of a polycationic carrier and a nucleic acid, in any physical form, for use in transfecting eukaryotic cells. A transfection complex may include additional moieties, e.g., targeting molecules such as receptor ligands or antibody fragments, or other accessory molecules. For example, nuclear localizing peptides may be included for facilitating transport of the polynucleotide to the cell nucleus. Kalderon et al., (1984) Cell 39:499-509; Chelsky et al., (1989) Mol. Cell Biol. 9:2487-2492; Dingwall & Laskey (1991) Trends Biochem. Sci. 16:478-481. Proteins or peptides may be included in the transfection complex to facilitate release of the transfection complex from the endosome after internalization. Raja-Walia et al., (1995) Hum. Gene Therap. 2:521-530; Bai et al., (1993) J. Virol. 67:5198-5205. In addition, enzymes involved in transcription and/or translation may be included to facilitate gene expression in the cell cytoplasm without transport to the cell nucleus. Gao & Huang (1993) Nucl Acids Res. 21:2867-2872.

Detailed Description Paragraph Right (17):
The transfection complexes may also be prepared to include a targeting moiety, to target delivery of the complex to the desired target cell in vivo. Thus, strategies are known in the art for including receptor ligands for delivery to cells expressing the appropriate receptor, or using antibodies or antibody fragments to target transfection complexes to cells expressing a specific cell surface molecule. See WO 96/37194; Ferkol et al., (1993) J. Clin. Invest. 92:2394-2400.

Detailed Description Paragraph Right (68):
Following preparation of the complexes, several physical and chemical parameters were tested to analyze the differences in the two methods. Among these tests were particle size, turbidity, zeta potential, pH, and DNA and lipid integrity tests (HPLC, thin layer chromatography, and agarose gel electrophoresis). There were no significant differences in the physical characteristics of the complexes. No significant difference in chemical composition or degradation was observed. The particle size range of the complexes made by the static mixer was tighter than those made by the diluter method as shown in Table 4. Importantly, complexes prepared by either method showed equivalent transfection efficiencies when tested in vivo by intraperitoneal injection of 250 .mu.l of complex into SKOV-3 tumor-bearing Balb/C nude mice. Tumors were remove 24 hours post injection and assayed for the presence of chloramphenicol acetyltransferase (CAT) reporter protein.

CLAIMS:

3. The method according to claim 1 wherein the polycation is selected from the group consisting of cationic lipid, polylysine, polyarginine, and polyhistidine.

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L5: Entry 21 of 23 File: DWPI Nov 9, 1999

DERWENT-ACC-NO: 2000-037288
DERWENT-WEEK: 200003

TITLE: Production of serum-stable lipid-encapsulated plasmid particles useful for transfecting cells in vitro or in vivo, especially for gene therapy

INVENTOR: BALLY, M B; CULLIS, P R ; HOPE, M ; WHEELER, J J

PRIORITY-DATA: 1995US-0484282 (June 7, 1995)

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
US 5981501 A	November 9, 1999		031	A61K048/00

INT-CL (IPC): A61 K 9/127; A61 K 48/00

ABSTRACTED-PUB-NO: US 5981501A
BASIC-ABSTRACT:

NOVELTY - Production of serum-stable particles that comprise a plasmid encapsulated in a lipid bilayer and have a diameter of 50-150 nm comprising combining a plasmid with cationic lipids in a detergent solution, contacting noncationic lipids with the mixture, and removing the detergent, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

(1) production of serum-stable particles that comprise a plasmid encapsulated in a lipid layer and have a diameter of 50-150 nm by preparing a mixture comprising cationic and noncationic lipids in an organic solvent, contacting an aqueous plasmid solution with the mixture to form a single phase, and removing the organic solvent; and

(2) methods for introducing a plasmid into a cell comprising contacting a cell with the plasmid-lipid particles above.

USE - The particles are useful for introducing the plasmid into cells, especially for gene therapy.

ADVANTAGE - The particles can be administered intravenously, are capable of carrying large amounts of DNA, protect the DNA against extracellular degradation, and are capable of transfecting target cells in such a way that the DNA is not digested intracellularly.

ABSTRACTED-PUB-NO: US 5981501A
EQUIVALENT-ABSTRACTS:

CHOSEN-DRAWING: Dwg.0/24

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L13: Entry 37 of 38 File: USPT Jul 21, 1998

DOCUMENT-IDENTIFIER: US 5783566 A
TITLE: Method for increasing or decreasing transfection efficiency

Brief Summary Paragraph Right (5):
Genes can also be introduced that make cells chemosensitive. In some cases, a disease is encoded by multiple, discontinuous genes. In this case, replacing a single gene is unlikely to lead to eradication of the disease. However, a "suicide" gene may be transfected to induce the self-destruction of diseased cells and tissues. This approach is currently being developed to treat a number of cancers. In one strategy, target tissues are transfected with the Herpes Simplex Virus-thymidine kinase (HSV-tk) gene and then treated with gancyclovir, a nucleotide analog. Phosphorylated gancyclovir produced by transfected cells is incorporated into genomic DNA and further elongation is prevented. This mode of treatment is enhanced by the "by-stander effect" i.e., the passage of phosphorylated gancyclovir into neighboring non-transduced cells through gap junctions. Thus, the growth of the tumor is inhibited by transfecting only a fraction of the tumor mass. In this type of gene transfer, expression can be either stable or transient.

Brief Summary Paragraph Right (36):
When the cationic species is a cationic liposome, the liposome can be prepared to specifically target a certain cell type. For example, certain antibodies are known to target liposomes to various cells. Tumor cells or ischemic tissue can be targeted by using unilamellar vesicles less than 200 nm in diameter.

Brief Summary Paragraph Right (41):
Transfection efficiency can also be controlled by modifying proteoglycan expression such that the cells express the various proteoglycans on the cell surface in a different ratio than untreated cells. In some cases, higher concentrations of chondroitan-based proteoglycans on the surface of cells causes increased transfection efficiency, whereas in others, higher concentrations of heparan sulfate-based proteoglycans on the surface of cells causes increased transfection efficiency. One of skill in the art can readily determine which proteoglycans are preferred. Methods for modulating the ratio of heparan sulfate to chondroitin sulfate using glycosaminoglycan biosynthesis inhibitors is known in the art, as described in Timar, et al., Int. J Cancer, 62:755-761 (1995). Suitable glycosaminoglycan biosynthesis inhibitors for use in the present invention include, but are not limited to, .beta.-D-xyloside, 2-deoxy-D-glucose, ethane-1-hydroxy-1,1-diphosphonate and 5-hexyl-2-deoxyuridine.

Detailed Description Paragraph Right (2):
The terms "polypeptide" and "protein" are used interchangeably. Suitable cell types that can be transfected using the methods described herein include, but are not limited to, fibroblasts, myoblasts, hepatocytes, cells of hematopoetic origin such as white blood cells and bone marrow cells, cancer cells and ischemic tissue. Transfection can be performed in vitro, ex vivo, or in vivo. The genetic material can be transiently expressed or stably expressed.

Detailed Description Paragraph Right (13):
Suitable cationic lipids for use in the present invention include, but are not limited to, DOTMA, Lipofectin (GIBCO/BRL, Gaithersburg, Md.), 1,2-bis(oleoyloxy)3-(trimethylammonio)propane (DOTAP), N-(w, w-1-dialkoxy)-alkyl-N,N,N-trisubstituted ammonium surfactants, complex cationic lipids having similar structures and properties and mixtures of these. Particularly preferred cationic lipids are those cationic lipids that are readily degradable in vivo. These include analogs of DORI (DL-1,2-dioleoyl-3-dimethylaminopropyl-.beta.-hydroxyethylammonium) and DORIE

(DL-1,2-O-dioleyl-3-dimethylaminopropyl-.beta.-hydroxyethylammonium) as well as DORI ester/ether compounds (DL-1-O-oleyl-2-oleoyl-3-dimethylaminopropyl-.beta.-hydroxyethylammonium or DL-1-oleoyl-2-O oleyl-3-dimethyl-aminopropyl-.beta.-hydroxyethylammonium).

Detailed Description Paragraph Right (22):
Cytokines are polypeptide mediators which can be produced by a variety of cells. Cytokines such as interleukin 3 mediate proteoglycan expression on the surface of cells (Nietfeld, Experientia 49:456-469 (1993). Suitable cytokines for use in practicing the present invention include, but are not limited to, epidermal growth factor (EGF), fibroblast growth factor (FGF), granulocyte colony stimulating factor (GCSF), granulocyte macrophage colony stimulating factor (GMC-SF), interferon gamma (IG), insulin-like growth factor-1 (ILGF), interleukin-1 alpha (IL-1 alpha), interleukin-1 beta (IL-1 beta), interleukin-1 receptor antagonist (IL-1 RA), interleukin-3 (IL-3), interleukin-4 (IL-4), interleukin-5 (IL-5), interleukin-6 (IL-6), macrophage colony stimulating factor (MCSF), platelet derived growth factor (PDGF), transforming growth factor beta (TGF beta), and tumor necrosis factor alpha (TNF alpha).

Detailed Description Paragraph Right (27):
Transient expression of a gene product can be advantageously used to promote an immune response. Thus, viral diseases can be treated by interferon expression and cytokines can be used to stimulate the immune system to react against foreign antigens or cancers. Similarly, foreign proteins can be expressed transiently from target cells to generate an immune response.

Detailed Description Paragraph Right (51):
Liposomes are microscopic delivery vesicles formed when amphiphilic lipids are mixed with water (i.e., hydrated), and include one or more spherical lipid bilayers which surround an internal aqueous phase. Amphiphilic lipids dispersed in aqueous solution spontaneously form bilayers with the hydrocarbon tails directed inward and the polar headgroups outward to interact with water. Simple agitation of the mixture generally produces multilamellar liposomes (MLVs), structures with many bilayers in an onion-like form. MLVs typically have a mean diameter of between 1,000 to 10,000 nm. MLVs, mainly because they are relatively large, are usually taken up by the reticuloendothelial system (RES).

Detailed Description Paragraph Right (52):
Unilamellar liposomes (UVs) can be formed, for example, by sonicating a dispersion of MLVs. Typical sizes of UVs range from approximately 30-1000 nm. Preferably, the liposomes used in the present invention have a mean diameter less than 200 nm.

Detailed Description Paragraph Right (57):
Transfection efficiency can be increased by incorporating a lysophosphatide into the liposome formulation. The lysophosphatides can be present in amounts up to approximately a third of the total lipid concentration. Preferred lysophosphatides include Iysophosphatidylcholines such as I-oleoyllysophosphatidylcholine and lysophosphatidylethanolamines. Particularly preferred lysophosphatides are DOTMA, 1,2-bis(oleoyloxy)3-(trimethylammonio)propane (DOTAP), Lipofectin (GIBCO/BRL, Gaithersburg, Md.) and mixtures of these.

Detailed Description Paragraph Right (61):
The liposomes of the present invention can be targeted through various means. The size of the liposome provides one means for targeting the liposomes. For example, relatively small UVs efficiently target ischemic tissue and tumor tissue, as described in U.S. Pat. No. 5,527,538, and U.S. Pat. Nos. 5,019,369, 5,435,989 and 5,441,745 to Presant et al., the contents of which are hereby incorporated by reference.

Detailed Description Paragraph Right (62):
The liposomes can be targeted according to the mode of administration. For example, lung tissue can be targeted by intranasal administration, cervical cells can be targeted by intravaginal administration, and prostate tumors can be targeted by intrarectal administration. Skin cancer can be targeted by topical administration. Depending on location, tumors can be targeted by injection into the tumor mass.

Detailed Description Paragraph Right (63):
Further, liposomes can be targeted by incorporating a ligand such as an antibody, a receptor, or other compound known to target liposomes to various sites, into the liposomal formulation. The ligands can be attached to cationic lipids used to form the liposomes, or to a neutral lipid such as cholesterol used to stabilize the liposome.

Ligands that are specific for one or more specific cellular receptor sites are attached to a vesicle to form a delivery vehicle that can be targeted with a high degree of specificity to a target cell population of interest.

Detailed Description Paragraph Right (64):
Suitable ligands for use in the present invention include, but are not limited to, sugars, proteins such as antibodies, hormones, lectins, major histocompatibility complex (MHC), and oligonucleotides that bind to or interact with a specific site. An important criteria for selecting an appropriate ligand is that the ligand is specific and is suitably bound to the surface of the vesicles in a manner which preserves the specificity. For example, the ligand can be covalently linked to the lipids used to prepare the liposomes. Alternatively, the ligand can be covalently bound to cholesterol or another neutral lipid, where the ligand-modified cholesterol is used to stabilize the lipid bilayer.

Detailed Description Paragraph Right (65):
Cytokines are preferred ligands. IL-2 is a preferred cytokine. This ligand is particularly useful for targeting specific activated T- and B-cell populations in view of the particular specificity for the high affinity IL-2 receptors on such cells. The IL-2 ligand can be stabilized for in vivo use by certain amino acid substitutions, as described in Wang and Mark, Science 224:1431 (1984). Other suitable cytokines for use as ligands in the present invention include, but are not limited to, antigrowth factors, B cell growth factors, chalcones, chemotactic factors, colony stimulating factor, GM-CSF, G-CSF, growth factors, interferon alpha and beta, interleukin-1 alpha and beta, Interleukins 1-6, lymphotoxin, tumor necrosis factor, macrophage inhibitory factors, and T-cell replacing factor.

Detailed Description Paragraph Right (66):
Some lipids already include ligands that are suitable for targeting various cell types. These include glycolipids, lipoproteins, glycoproteins, and hydrophobic proteins. Examples described in the literature include gangliosides (Jonah, et al., Biochem. Biophys. Acta 541:321 (1978)), lactosyl ceramide (Spanjer and Scherphof, Biochem. Biophys. Acta, 734:40 (1983)), and sialoglycoprotein (Takada et al., Biochem. Biophys. Acta, 802:237 (1984). Synthetic cholesterol derivatives covalently bound to sugars such as aminomannose have been described, for example, in Mauk, et al., Science 207:309 (1980). Vesicles including aminomannose derived cholesterol have been demonstrated to target EmT6 tumor cells. These compounds can be incorporated into the lipid bilayer when the liposomes are prepared. Dinitrophenyl caproylphosphatidylethanolamine and other phosphatidylethanolamine derivatives linking small peptides have also been directly incorporated into lipid bilayers. Proteins have been covalently linked to liposomes through thiol, hydroxy and/or amine groups on the protein and the lipid, using known coupling techniques, for example, carbodiimide or glutaraldehyde chemistry.

Detailed Description Paragraph Right (67):
It is preferred that the ligand be covalently bound to the surface of a preformed lipid vesicle, to ensure that the ligand is present on the outside of the vesicle. The binding can occur directly or through a suitable linker molecule.

Detailed Description Paragraph Right (72):
A biopsy or cell sample is preferably obtained from a patient in need of gene therapy. Alternatively, the treated cells can be derived from a secondary source such as a cell line or a cell donor. The cell sample is preferably obtained from a tissue where gene expression would be most advantageous. The cell sample may be derived from a variety of tissues, including, but not limited to, skin, liver, pancreas, spleen, muscle, bone marrow, nervous system cells, blood cells, and tumor cells.

Detailed Description Paragraph Right (86):
Methods to return treated cancer cells to a patient will depend on the cancer type. One can inject autologous transfected cancer cells into either a tumor mass or bone marrow. One can stimulate immune responses directed to both the treated and untreated tumor cells, thereby reducing or clearing the tumor mass, by expressing an MHC protein, a foreign protein or an immune stimulating protein. Other transfected cancer cells of hematopoietic origin may be introduced directly into the blood stream. Cells from hard tissue tumors may be replaced directly into a tumor mass to elicit tumor regression through external injection or internal injection using catheters or the like.

Detailed Description Paragraph Right (90):
Preferably, the cationic species used to prepare the complex is a cationic liposome.

More preferably, the liposome is prepared to specifically target a certain cell type. For example, certain antibodies are known to target liposomes to various cells. By using unilamellar vesicles less than 200 nm in diameter, tumor cells can be targeted for transfection. By administering the liposomes intranasally via an aerosol, lung tissue can be targeted.

Detailed Description Paragraph Right (95):
Transfection efficiency can also be controlled by modifying proteoglycan expression such that the cells express a different ratio of different types of proteoglycans on the cell surface. In some cases, higher concentrations of chondroitan-based proteoglycans on the surface of cells causes increased transfection efficiency, whereas in others, higher concentrations of heparan sulfate-based proteoglycans on the surface of cells causes increased transfection efficiency. One of skill in the art can readily determine which proteoglycans are preferred. Methods for modulating the ratio of heparan sulfate to chondroitin sulfate using glycosaminoglycan biosynthesis inhibitors is known in the art, as described by Timar, J. et al. in Int. J. Cancer, 62:755-761 (1995). Suitable glycosaminoglycan biosynthesis inhibitors for use in the present invention include, but are not limited to, .beta.-D-xyloside, 2-deoxy-D-glucose, ethane-1-hydroxy-1,1-diphosphonate and 5-hexyl-2-deoxyuridine.

Detailed Description Paragraph Right (102):
Cancer cells also exhibit low cell surface contentrations of proteoglycans. Although these cells can be transfected, the transfection efficiency can be increased by increasing the cell surface concentration of proteoglycans.

Detailed Description Paragraph Left (20):
Transfection of Non-Differentiated Cells and Cancer Cells

Other Reference Publication (13):
Timar et al., Int. J. Cancer 62:755-761.

CLAIMS:

2. The method of claim 1, wherein the cationic species is selected from the group consisting of cationic lipids, cationic liposomes, calcium ions, lipopolyamine, polyethylene imine, polycationic amphiphiles, DEAE-dextran and dendrite polymers containing cationic functional groups.

6. The method of claim 3, wherein the liposomes comprise a lipid bound to a ligand, wherein the lipid is selected from the group consisting of neutral phospholipids, cationic phospholipids, cationic lipids, neutral lipids, and lysolipids, and wherein the ligand is selected from the group consisting of sugars, proteins, hormones, cytokines, lectins, major histocompatibility complex (MHC) and oligonucleotides that bind to or interact with a specific site.

8. The method of claim 1, wherein the cells to be transfected are selected from the group consisting of fibroblasts, myoblasts, hepatocytes, cells of hematopoetic origin, cancer cells, ischemic tissue, cells neurons and other cells of the nervous system and non-differentiated cells.

9. The method of claim 1, wherein the cells to be transfected are non-differentiated cells or cancer cells.

24. The method of claim 1 wherein the genetic material operatively codes for interferon and cytokines are administered to stimulate the immune system to react against foreign antigens or cancers.

25. The method of claim 1, wherein the genetic material encodes a protein that stimulates the immune system to recognize a given population of cancer cells as foreign.

26. The method of claim 1, wherein the genetic material renders cancer cells chemosensitive.

37. The method of claim 3, wherein the cationic lipid is selected from the group consisting of DOTMA (N-[1-(2,3-dioleyloxy)propyl]-N,N,N-trimethylammonium), Lipofectin, 1,2-bis(oleoyloxy)-3-(trimethylammonio)propane (DOTAP), N-(w,w-1-dialkoxy)-alkyl-N,N,N-trisubstituted ammonium surfactants, DORI (DL-1,2-dioleoyl-3-dimethylaminopropyl-.beta.-hydroxyethylammonium), DORIE

(DL-1,2-O-dioleyl-3-dimethylaminopropyl-.beta.-hydroxyethylammonium), DORI ester/ether compounds (DL-1-O-oleyl-2-oleoyl-3-dimethylaminopropyl-.beta.-hydroxyethylammonium, DL-1-oleoyl-2-O-oleyl-3-dimethylaminopropyl-.beta.-hydroxyethylammonium) and mixtures thereof.

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L13	L12 and l2	38	L13
L12	L11 and l10	51	L12
L11	tumor or cancer	149545	L11
L10	L9 and l3	53	L10
L9	l8 and l4	75	L9
L8	L7 with l1	248	L8
L7	polylysine or polycationic	6962	L7
L6	L5 same l2	6	L6
L5	l4 with l1	23	L5
L4	diameter with (nanometer or nm)	25073	L4
L3	folate or transferin or ligand	80680	L3
L2	DOTAP or DDAB	807	L2
L1	cationic lipid or cationic amphiphile or cationic liposome	3785	L1

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L6: Entry 4 of 6 File: USPT Dec 14, 1999

US-PAT-NO: 6001644
DOCUMENT-IDENTIFIER: US 6001644 A

TITLE: Mammalian transformation complex comprising a lipid carrier and DNA encoding CFTR

DATE-ISSUED: December 14, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Debs; Robert J.	Mill Valley	CA		
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US-CL-CURRENT: 435/320.1; 128/203.22, 424/450, 435/458, 514/44, 600/243, 600/249, 601/1

CLAIMS:

What is claimed is:

1. A mammalian transformation complex, comprising:

a lipid carrier, comprising cationic lipid and a non-cationic lipid, wherein the complex is between about 100 nanometers and 10 microns in diameter; and

a DNA expression cassette comprising a promoter in operable linkage with a polynucleotide encoding a functional cystic fibrosis transmembrane conductance regulator (CFTR), wherein said DNA expression cassette is in a ratio of less than 4:1 micrograms DNA to nanomoles cationic lipid; and

wherein said complex does not aggregate in vitro.

2. The mammalian transformation complex of claim 1, wherein the non-cationic lipid is a steroid.

3. The mammalian transformation complex of claim 1, wherein the non-cationic lipid is cholesterol.

4. The mammalian transformation complex of claim 1, wherein the cationic lipid and non-cationic lipid are in a molar ratio of about 1:1.

5. The mammalian transformation complex of claim 1, wherein the lipid carrier is an MLV (multilamellar vesicle).

6. The mammalian transformation complex of claim 1, wherein the lipid carrier is an SUV (small unilamellar vesicle).

7. The mammalian transformation complex of claim 1, wherein the promoter is an inducible promoter.

8. The mammalian transformation complex of claim 1 wherein the promoter is from a cystic fibrosis transmembrane conductance regulator gene.

9. The mammalian transformation complex of claim 1, wherein the complex comprises DOPE and a lipid selected from the group consisting of DOTMA, DDAB, DOTAP and L-PE.

10. The mammalian transformation complex of claim 1, wherein the complex comprises cholesterol and a lipid selected from the group consisting of DOTMA, DDAB, DOTAP, E-PC, CEBA, E-DMPC, L-PE, and DOPE.

11. The mammalian transformation complex of claim 1, wherein the complex comprises a lipid selected from the group consisting of cholesterol, DOTMA, DDAB, DOTAP, E-PC, CEBA, E-DMPC, L-PE, and DOPE.

12. The mammalian transformation complex of claim 1, wherein the complex comprises a lipid other than DOTMA.

13. The mammalian transformation complex of claim 1, wherein the complex does not comprise DOTMA.

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L5: Entry 4 of 23 File: USPT Aug 7, 2001

DOCUMENT-IDENTIFIER: US 6271206 B1
TITLE: Sonic nebulized nucleic acid/cationic liposome complexes and methods for pulmonary gene delivery

Detailed Description Paragraph Right (9):
Nucleic acid and stabilizing agents may be formulated into a complex using any technique appropriate for the particular components. Such techniques are well known to those of ordinary skill in the art. In complexes containing one or more lipid stabilizing agents, large multilamellar vesicles (MLVs) or extruded liposomes (i.e., unilamellar vesicles) may be prepared prior to the addition of nucleic acid. For example, cationic liposomes composed of DOTMA, along with co-lipids DOPE or cholesterol at a 1:1 mole ratio, may be extruded through polycarbonate filters with different pore diameters (such as 200, 400 and 800 nm) to prepare unilamellar vesicles of different size. Small unilamellar vesicles (SUVs), which have diameters less than 100 nm, may also be prepared by extrusion through appropriately sized filters. Plasmid/lipid complexes may then be prepared by controlled mixing of the cationic liposomes with plasmid DNA at a pre-determined ratio (the stoichiometry of DNA to cationic lipids depends on charge and concentration) using a continuous infusion apparatus. Preferably, the complexes are prepared in 10% lactose so that the formulation is isotonic. Liposomes and plasmids may be delivered to the mixing chamber at a precise rate using, for example, a variable flow self-priming peristaltic pump (e.g., VWR Model Number 54856 (VWR, Houston, Tex.). Steady state conditions may be maintained in the mixing chamber such that the input flow rate of the components into the mixing chamber is equal to the output rate of the formulated complex from the mixing chamber. The complexation efficiency (ie., the fraction of plasmid condensed onto the cationic lipid) may be determined by agarose gel electrophoresis. Plasmid integrity may also be determined using agarose gel electrophoresis, by stripping the DNA from the complex with Triton X, and comparing the stripped DNA bands with that of a naked DNA control.

Detailed Description Paragraph Right (19):
A plasmid containing the bacterial reporter gene chloramphenicol acetyl transferase (CAT) driven by the cytomegalovirus (CMV) promoter/enhancer was constructed using standard techniques. Unilamellar vesicles composed of the cationic lipid DOTMA with either DOPE or cholesterol (Chol) (all lipids obtained from Avanti Polar Lipids Inc., Alabaster, Ala.) at a 1:1 mole ratio were prepared by extrusion through filters (Poretics Corp., Livermore, Calif.) with pore diameters of 200, 400 and 800 nm.

Detailed Description Paragraph Right (31):
Cationic lipids and co-lipids were formulated as large multilamellar vesicles (MLVs) or unilamellar vesicles prepared by extrusion through polycarbonate membrane filters with varying pore diameters (100, 400 or 800 nm). CMV-CAT/DOTMA:Chol complexes at varying charge ratios were formulated in 10% (w/v) lactose as described in Example 1.

Detailed Description Paragraph Right (34):
Cationic lipids and co-lipids were formulated as large multilamellar vesicles (MLVs) or extruded through polycarbonate membrane filters with varying pore diameters (100, 400 and 800 nm). CMV-CAT/DOTMA:Chol and CMV-CAT/DOTMA:DOPE complexes with varying particle diameters and a fixed charge ratio of 1:3 (-:+) were formulated in 10% (w/v) lactose as described in Example 1. The plasmid DNA concentration was 125 .mu.g/mL. The mean diameter and zeta potential of the complexes were characterized by dynamic light scattering and Doppler electrophoretic light scattering and the complexation efficiency was determined by agarose gel electrophoresis, as described above.

CLAIMS:

7. The method of claim 1 wherein the cationic liposomes have a diameter equal to or greater than about 100 nm prior to complexing with the nucleic acid.

8. The method of claim 1 wherein the cationic liposomes have a diameter equal to or greater than about 200 nm prior to complexing with the nucleic acid.

9. The method of claim 1 wherein the cationic liposomes have a diameter equal to or greater than about 400 nm prior to complexing with the nucleic acid.

10. The method of claim 1 wherein the cationic liposomes have a diameter equal to or greater than about 800 nm prior to complexing with the nucleic acid.

15. The nucleic acid delivery appartus of claim 14 wherein the cationic liposomes have a diameter equal to or greater than about 100 nm prior to admixing with nucleic acid.

16. The nucleic acid delivery apparatus of claim 14 wherein the cationic liposomes have a diameter equal to or greater than about 200 nm prior to admixing with the nucleic acid.

17. The nucleic acid delivery apparatus of claim 14 wherein the cationic liposomes have a diameter equal to or greater than about 400 nm prior to admixing with the nucleic acid.

18. The nucleic acid delivery apparatus of claim 14 wherein the cationic liposomes have a diameter equal to or greater than about 800 nm prior to admixing with the nucleic acid.

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L5: Entry 11 of 23 File: USPT Oct 12, 1999

DOCUMENT-IDENTIFIER: US 5965542 A
TITLE: Use of temperature to control the size of cationic liposome/plasmid DNA complexes

Detailed Description Paragraph Right (6):
In view of the above surprising discovery, the present invention provides methods of preparing cationic liposome/nucleic acid complexes comprising combining a first solution of preformed cationic liposomes with a second solution of nucleic acid, wherein each of the first and second solutions have been pre-equilibrated to temperatures of from about 0.degree. C. to about 12.degree. C. and wherein the preformed cationic liposomes are unilamellar and have a mean diameter of from 100 to 150 nm.

CLAIMS:

1. A method of preparing cationic liposome/nucleic acid complexes comprising combining a first solution of preformed cationic liposomes with a second solution of nucleic acids, wherein each of said first and second solutions have been pre-equilibrated to temperatures of from 0.degree. C. to 12.degree. C., said preformed cationic liposomes being unilamellar, having a mean diameter of from 100 to 150 nm, and consisting essentially of unsaturated cationic lipids and neutral lipids selected from the group consisting of DOPE, cholesterol and combinations thereof.

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L13: Entry 7 of 38 File: PGPB Nov 8, 2001

PGPUB-DOCUMENT-NUMBER: 20010038851
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20010038851 A1

TITLE: Therapeutic liposome composition and method of preparation

PUBLICATION-DATE: November 8, 2001

INVENTOR-INFORMATION:

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Zalipsky, Samuel	Redwood City		US	

US-CL-CURRENT: 424/450; 424/142.1

CLAIMS:

It is claimed:

1. Reagents for use in preparing a therapeutic liposome composition sensitized to a target cell, said reagents comprising a liposomal composition composed of pre-formed liposomes having an entrapped therapeutic agent; and a plurality of conjugates, each conjugate composed of (i) a lipid having a polar head group and a hydrophobic tail, (ii) a hydrophilic polymer having a proximal end and a distal end, said polymer attached at its proximal end to the head group of the lipid, and (iii) a targeting ligand attached to the distal end of the polymer; wherein the therapeutic, target-cell sensitized liposome composition is formed by incubating the liposomal composition with a selected conjugate.

2. The composition of claim 1, wherein the targeting ligand is an antibody or an antibody fragment.

3. The composition of claim 2, wherein the antibody or antibody fragment is of mouse origin and is humanized to remove murine epitopes.

4. The composition of claim 2, wherein the targeting ligand specifically binds to an extracellular domain of a growth factor receptor.

5. The composition of claim 4, wherein the receptors are selected from the group consisting of c-erbB-2 protein product of the HER2/neu oncogene, epidermal growth factor receptor, basic fibroblast growth factor receptor, and vascular endothelial growth factor receptor.

6. The composition of claim 2, wherein the targeting ligand binds a receptor selected from the group consisting of E-selectin receptor, L-selectin receptor, P-selectin receptor, folate receptor, CD4 receptor, CD19 receptor, a: integrin receptors and chemokine receptors.

7. The composition of claim 1, wherein the targeting ligand is selected from the group consisting of folic acid, pyridoxal phosphate, vitamin B12, sialyl Lewis.sup.x, transferrin, epidermal growth factor, basic fibroblast growth factor, vascular endothelial growth factor, VCAM-1, ICAM-1, PECAM-1, RGD peptides and NGR peptides.

8. The composition of claim 1, wherein the targeting ligand binds a receptor on a malignant B-cell or T-cell, said receptor selected from the group consisting of CD19, CD20, CD22, CD4, CD7 and CD8.

9. The composition of claim 1, wherein the hydrophilic polymer is selected from the group consisting of polyvinylpyrrolidone, polyvinylmethylether, polymethyloxazoline, polyethyloxazoline, polyhydroxypropyloxazoline, polyhydroxypropylmethacrylamide, polymethacrylamide, polydimethylacrylamide, polyhydroxypropylmethacrylate, polyhydroxyethylacrylate, hydroxymethylcellulose, hydroxyethylcellulose, polyethyleneglycol, polyaspartamide and hydrophilic peptide sequences.

10. The composition of claim 1, wherein the hydrophilic polymer is polyethylene glycol.

11. The composition of claim 10, wherein the polyethylene glycol has a molecular weight between 500-5,000 daltons.

12. The composition of claim 1, wherein the liposomes further contain a cationic lipid.

13. The composition of claim 1, wherein the entrapped therapeutic agent is a cytotoxic drug.

14. The composition of claim 13, wherein the cytotoxic drug is an anthracycline antibiotic selected from the group consisting of doxorubicin, daunorubicin, epirubicin and idarubicin and analogs thereof.

15. The composition of claim 13, wherein the cytotoxic agent is a platinum compound selected from cisplatin, carboplatin, ormaplatin, oxaliplatin, zeniplatin, enloplatin, lobaplatin, spiroplatin, ((-)-(R)-2-aminomethylpy- rrolidine (1,1-cyclobutane dicarboxylato)platinum), (SP-4-3(R)-1,1-cyclobutane-dicarboxylato(2-)-(2-methyl-1, 4-butanediamine-N.sub.1N')platinum), nedaplatin and (bis-acetato-ammine-dichloro-cyclohexylamine-platinum(IV)).

16. The composition of claim 13, wherein the cytotoxic agent is a topoisomerase 1 inhibitor selected from the group consisting of topotecan, irinotecan, (7-(4-methylpiperazino-methylene)-10,11-ethylenedi- oxy-20(S)-camptothecin), 7-(2-(N-isopropylamino)ethyl)-(20S) -camptothecin, 9-aminocamptothecin and 9-nitrocamptothecin.

17. The composition of claim 13, wherein the cytotoxic agent is a vinca alkaloid selected from the group consisting of vincristine, vinblastine, vinleurosine, vinrodisine, vinorelbine and vindesine.

18. The composition of claim 1, wherein the entrapped agent is a nucleic acid.

19. The composition of claim 18, wherein the nucleic acid is an antisense oligonucleotide or ribozyme.

20. The composition of claim 18, wherein the nucleic acid is a plasmid containing a therapeutic gene which when internalized by the target cells achieves expression of the therapeutic gene to produce a therapeutic gene product.

21. A plurality of targeting conjugates for use in preparing a targeted, therapeutic liposome composition, each conjugate composed of a (i) a lipid having a polar head group and a hydrophobic tail, (ii) a hydrophilic polymer having a proximal end and a distal end, said polymer attached at its proximal end to the head group of the lipid, and (iii) a targeting ligand attached to the distal end of the polymer.

22. The conjugates of claim 21, wherein the lipid is selected from the group consisting of distearoyl phosphatidylethanolamine, distearoyl-phosphatidylcholine, monogalactosyl diacylglycerols and digalactosyl diacylglycerols.

23. The conjugates of claim 21, wherein the hydrophilic polymer is selected from the group consisting of polyvinylpyrrolidone, polyvinylmethylether, polymethyloxazoline, polyethyloxazoline, polyhydroxypropyloxazoline, polyhydroxypropylmethacrylamide, polymethacrylamide, polydimethylacrylamide, polyhydroxypropylmethacrylate- , polyhydroxyethylacrylate, hydroxymethylcellulose, hydroxyethylcellulose, polyethyleneglycol, polyaspartamide and hydrophilic peptide sequences.

24. The conjugates of claim 21, wherein the hydrophilic polymer is polyethylene glycol.

25. The conjugates of claim 24, wherein the polyethylene glycol has a molecular weight between 500-5,000 daltons.

26. The conjugates of claim 21, wherein the targeting ligand is an antibody or an antibody fragment.

27. The conjugates of claim 26, wherein the antibody or antibody fragment is of mouse origin and is humanized to remove murine epitopes.

28. The conjugates of claim 21, wherein the targeting ligand specifically binds to an extracellular domain of a growth factor receptor.

29. The conjugates of claim 28, wherein the receptors are selected from the group consisting of c-erbB-2 protein product of the HER2/neu oncogene, epidermal growth factor receptor, basic fibroblast growth factor receptor, and vascular endothelial growth factor receptor.

30. The conjugates of claim 21, wherein the targeting ligand binds a receptor selected from the group consisting of E-selectin receptor, L-selectin receptor, P-selectin receptor, folate receptor, CD4 receptor, CD19 receptor, .alpha..beta. integrin receptors and chemokine receptors.

31. The conjugates of claim 21, wherein the targeting ligand binds a receptor on a malignant B-cell or T-cell, said receptor selected from the group consisting of CD19, CD20, CD22, CD4, CD7 and CD8.

32. The conjugates of claim 21, wherein the targeting ligand is selected from the group consisting of folic acid, pyridoxal phosphate, vitamin B12, sialyl Lewis.sup.x, transferrin, epidermal growth factor, basic fibroblast growth factor, vascular endothelial growth factor, VCAM-1, ICAM-1, PECAM-1, RGD peptides and NGR peptides.

33. A method of formulating a therapeutic liposome composition having sensitivity to a target cell, comprising selecting a liposomal composition composed of pre-formed liposomes having an entrapped therapeutic agent; selecting from a plurality of targeting conjugates a targeting conjugate composed of (i) a lipid having a polar head group and a hydrophobic tail, (ii) a hydrophilic polymer having a proximal end and a distal end, said polymer attached at its proximal end to the head group of the lipid, and (iii) a targeting ligand attached to the distal end of the polymer; and combining the selected liposome formulation and the selected targeting conjugate to form said therapeutic, target-cell sensitive liposome composition.

34. The method of claim 33, wherein said combining includes incubating under conditions effective to achieve insertion of the selected targeting conjugate into the liposomes of the selected liposome formulation.

35. The method of claim 33, wherein said selecting a liposome formulation includes determining the sensitivity of the target cell to the therapeutic activity of the entrapped therapeutic agent.

36. The method of claim 33, wherein said selecting a targeting conjugate includes determining the ability of the targeting ligand to bind cell surface receptors expressed on the target cell.

37. The method of claim 36, wherein said selecting a targeting conjugate is based on (i) the ability of a targeting ligand to bind to cell surface receptors expressed on the target cell and (ii) the ability of the target cell to internalize liposomes bound to the target cell by binding between the target cell and the targeting ligand.

38. The method of claim 33, wherein the targeting ligand is an antibody or an antibody fragment.

39. The method of claim 38, wherein the antibody or antibody fragment is of mouse origin and is humanized to remove murine epitopes.

40. The method of claim 38, wherein the targeting ligand specifically binds to an extracellular domain of a growth factor receptor.

41. The method of claim 40, wherein the receptors are selected from the group

consisting of c-erbB-2 protein product of the HER2/neu oncogene, epidermal growth factor receptor, basic fibroblast growth factor receptor, and vascular endothelial growth factor receptor.

42. The method of claim 38, wherein the targeting ligand binds a receptor selected from the group consisting of E-selectin receptor, L-selectin receptor, P-selectin receptor, folate receptor, CD4 receptor, CD19 receptor, .alpha..beta. integrin receptors and chemokine receptors.

43. The method of claim 33, wherein the targeting ligand binds a receptor on a malignant B-cell or T-cell, said receptor selected from the group consisting of CD19, CD20, CD22, CD4, CD7 and CD8.

44. The method of claim 33, wherein the targeting ligand is selected from the group consisting of folic acid, pyridoxal phosphate, vitamin B12, sialyl Lewis.sup.x, transferrin, epidermal growth factor, basic fibroblast growth factor, vascular endothelial growth factor, VCAM-1, ICAM-1, PECAM-1, RGD peptides and NGR peptides.

45. The method of claim 33, wherein the hydrophilic polymer is selected from the group consisting of polyvinylpyrrolidone, polyvinylmethylether, polymethyloxazoline, polyethyloxazoline, polyhydroxypropyloxazoline, polyhydroxypropylmethacrylamide, polymethacrylamide, polydimethylacrylamide, polyhydroxypropylmethacrylate, polyhydroxyethylacrylate, hydroxymethylcellulose, hydroxyethylcellulose, polyethyleneglycol, polyaspartamide and hydrophilic peptide sequences.

46. The method of claim 33, wherein the hydrophilic polymer is polyethylene glycol.

47. The method of claim 46, wherein the polyethylene glycol has a molecular weight between 500-5,000 daltons.

48. The method of claim 33, wherein the liposomes further contain a cationic lipid.

49. The method of claim 33, wherein the entrapped therapeutic agent is a cytotoxic drug.

50. The method of claim 49 wherein the cytotoxic drug is an anthracycline antibiotic selected from the group consisting of doxorubicin, daunorubicin, epirubicin and idarubicin and analogs thereof.

51. The method of claim 49, wherein the cytotoxic agent is a platinum compound selected from cisplatin, carboplatin, ormaplatin, oxaliplatin, zeniplatin, enloplatin, lobaplatin, spiroplatin, ((-)-(R)-2-aminomethylpy- rrolidine (1,1-cyclobutane dicarboxylato)platinum), (SP-4-3(R)-1,1-cyclobutane-dicarboxylato(2-)-(2-methyl-1,4-butanediamine-N,N')platinum), nedaplatin and (bis-acetato-ammine-dichloro-cyclohecylami- ne-platinum (IV)).

52. The method of claim 49, wherein the cytotoxic agent is a topoisomerase 1 inhibitor selected from the group consisting of topotecan, irinotecan (7-(4-methylpiperazino-methylene)-10,11-ethylenedioxy-20(S)-camptothecin)- , 7-12-(N-isopropylamino)ethyl)-(20S)-camptothecin, 9-aminocamptothecin and 9-nitrocamptothecin.

53. The method of claim 49, wherein the cytotoxic agent is a vinca alkaloid selected from the group consisting of vincristine, vinblastine, vinleurosine, vinrodisine, vinorelbine and vindesine.

54. The method of claim 33, wherein the entrapped agent is a nucleic acid.

55. The method of claim 54, wherein the nucleic acid is an antisense oligonucleotide or ribozyme.

56. The method of claim 54, wherein the nucleic acid is a plasmid containing a therapeutic gene which when internalized by the target cells achieves expression of the therapeutic gene to produce a therapeutic gene product.

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L13: Entry 10 of 38 File: USPT Jul 9, 2002

DOCUMENT-IDENTIFIER: US 6417326 B1
TITLE: Fusogenic liposomes

Detailed Description Paragraph Right (5):
The term "cationic lipid" refers to any of a number of lipid species which carry a net positive charge at physiological pH. Such lipids include, but are not limited to, DODAC, DOTMA, DDAB, DOTAP, DC-Chol and DMRIE. Additionally, a number of commercial preparations of cationic lipids are available which can be used in the present invention. These include, for example, LIPOFECTIN.RTM. (commercially available cationic liposomes comprising DOTMA and DOPE, from GIBCO/BRL, Grand Island, N.Y., USA); LIPOFECTAMINE.RTM. (commercially available cationic liposomes comprising DOSPA and DOPE, from GIBCO/BRL); and TRANSFECTAM.RTM. (commercially available cationic lipids comprising DOGS in ethanol from Promega Corp., Madison, Wis., USA).

Detailed Description Paragraph Right (16):
Examples of suitable cationic lipids include, but are not limited to, the following: DC-Chol, 3.beta.-(N-(N',N'-dimethylaminoethane)carbamoyl)cholesterol (see, Gao, et al., Biochem. Biophys. Res. Comm. 179:280-285 (1991); DDAB, N,N-distearyl-N,N,N-dimethylammonium bromide; DMRIE, N-(1,2-dimyristyloxyprop-3-yl)-N,N-dimethyl-N-hydroxyethyl ammonium bromide; DODAC, N,N-dioleyl-N,N-dimethylammonium chloride (see, commonly owned U.S. patent application Ser. No. 08/316,399, filed Sep. 30, 1994, which is incorporated herein by reference); DOGS, diheptadecylamidoglycyl spermidine; DOSPA, N-(1-(2,3-dioleyloxy)propyl)-N-(2-(sperminecarboxamido)ethyl)-N,N-dimethyl ammonium trifluoroacetate; DOTAP, N-(1-(2,3-dioleoyloxy)propyl)-N,N,N-trimethylammonium chloride; and DOTMA, N-(1-(2,3-dioleyloxy)propyl)-N,N,N-trimethylammonium chloride. In a presently preferred embodiment, N,N-dioleoyl-N,N-dimethylammonium chloride is used in combination with a phosphatidylethanolamine.

Detailed Description Paragraph Right (61):
As shown in FIG. 52, the first basic monomeric unit (1) can be used to generate the oligomer with the structure [Glu-TEG].sub.n (n=any number), whereas the second basic unit (2) can be used to generate the oligomer with the structure [Glu-TEG-Glu].sub.n (n=any number) shown in FIG. 53. It should be noted that in both sequences, doubling of the chain length can be performed in one reaction and this can be repeated according to the fmal size of the oligomer required in order to provide the desired properties and characteristic when applied to the transmembrane carrier system. Therefore, the number of steps to obtain a long chain is reduced significantly. Furthermore, the fusogenic oligomers of the present invention are designed such that they can be readily conjugated to a lipid anchor at one terminal end and/or to a targeting ligand or other factor at the other terminal.

Detailed Description Paragraph Right (71):
Cationic lipids useful in producing lipid based carriers for gene and oligonucleotide delivery include, but are not limited to, 3.beta.-(N-(N',N'-dimethylaminoethane)carbamoyl)cholesterol (DC-Chol); N,N-distearyl-N,N-dimethylammonium bromide (DDAB); N-(1,2-dimyristyloxyprop-3-yl)-N,N-dimethyl-N-hydroxyethyl ammonium bromide (DMRIE); diheptadecylamidoglycyl spermidine (DOGS); N-(1-(2,3-dioleyloxy)propyl)-N-(2-(sperminecarboxamido)ethyl)-N,N-dimethyl ammonium trifluoroacetate (DOSPA); N-(1-(2,3-dioleoyloxy)propyl)-N,N,N-trimethylammonium chloride (DOTAP); N-(1-(2,3-dioleyloxy)propyl)-N,N,N-trimethylammonium chloride (DOTMA); N,N-dioleyl-N,N-dimethylammonium chloride (DODAC); LIPOFECTIN, a commercially available cationic lipid comprising DOTMA and DOPE (GIBCO/BRL, Grand Island, N.Y.) (U.S. Pat. Nos. 4,897,355; 4,946,787; and 5,208,036 issued to Epstein, et al.);

LIPOFECTACE or DDAB (dimethyldioctadecyl ammonium bromide) (U.S. Pat. No. 5,279,883 issued to Rose); LIPOFECTAMINE, a commercially available cationic lipid composed of DOSPA and DOPE (GIBCO/BRL, Grand Island, N.Y.); TRANSFECTAM, a commercially available cationic lipid comprising DOGS (Promega Corp., Madison, Wis.).

Detailed Description Paragraph Right (75):
In certain embodiments of the present invention, it is desirable to target the liposomes of the invention using targeting moieties that are specific to a particular cell type, tissue, and the like. Targeting of liposomes using a variety of targeting moieties (e.g., ligands, receptors and monoclonal antibodies) has been previously described (see, e.g., U.S. Pat. Nos. 4,957,773 and 4,603,044, both of which are incorporated herein by reference).

Detailed Description Paragraph Right (76):
Examples of targeting moieties include monoclonal antibodies specific to antigens associated with neoplasms, such as prostate cancer specific antigen. Tumors can also be diagnosed by detecting gene products resulting from the activation or overexpression of oncogenes, such as ras or c-erB2. In addition, many tumors express antigens normally expressed by fetal tissue, such as the alphafetoprotein (AFP) and carcinoembryonic antigen (CEA). Sites of viral infection can be diagnosed using various viral antigens such as hepatitis B core and surface antigens (HBVc, HBVs) hepatitis C antigens, Epstein-Barr virus antigens, human immunodeficiency type-1 virus (HIV1) and papilloma virus antigens. Inflammation can be detected using molecules specifically recognized by surface molecules which are expressed at sites of inflammation such as integrins (e.g., VCAM-1), selectin receptors (e.g., ELAM-1) and the like.

Detailed Description Paragraph Right (88):
Another approach involves promoting leakage of liposome contents by heating a liposomal saturated target site above a critical temperature range, for example, by radio frequency heating of target tissues. Yatvin, et al., Science, 202:1290 (1978). Another approach has used liposomes prepared from pH-sensitive lipids, which leak their pharmaceutical contents into low pH target regions. Such areas of localized acidity are sometimes found in tumors; hence, it has been proposed that intravenous administration of such liposomes would preferably selectively release anti-cancer chemotherapeutic agents at target tumors (see, e.g., Yatvin, et al., Science, 210:1253 (1980)). A pH sensitive lipid is defined herein as a lipid that undergoes a chemical or conformational change upon exposure to a decreased pH.

Detailed Description Paragraph Right (104):
Other lipids can also be added to the virosome membranes during preparation. Fusion activity of the virosomes is optimally maintained when lipids similar to those of viral origin or lipid mixtures which closely resemble the lipid composition of the viral envelope are added. These lipids comprise cholesterol and phospholipids such as phosphatidylcholine (PC), sphingomyelin (SPM), phosphatidylethanolamine (PE), and phosphatidylserine (PS). However, other phospholipids may also be added. These include, but are not limited to, phosphatidylglycerol (PG), phosphatidic acid (PA), cardiolipin (CL), and phosphatidylinositol (PI), with varying fatty acyl compositions and of natural and/or (semi)synthetic origin, and dicetyl phosphate. Ceramide and various glycolipids, such as cerebrosides or gangliosides, may also be added. Cationic lipids may also be added, e.g., for concentrating nucleic acids in the virosomes and/or for facilitating virosome-mediated delivery of nucleic acids to cells. These include DOTMA, DOTAP (N-[1-(2,3-dioleoyloxy)propyl]-N,N,N-trimethylammonium chloride), DODAC (N,N-dioleyl-N,N, dimethylammonium chloride), DDAB and stearylamine or other aliphatic amines and the like. DODAC is a preferred cationic lipid for complexing nucleic acids to the virosome and the ensuing delivery of nucleic acids to cells, and is described in copending application U.S. Ser. No. 08/316,399, incorporated herein by reference. Particularly preferred concentrations of DODAC range from 25-45% (mol % of total phospholipids in the virus), more preferably 30-40%, and most preferably about 30% for the delivery of a nucleic acid such as DNA or antisense RNA to a cell. Additional lipids which may be suitable for use in the virosomes of the present invention are well known to persons of skill in the art. Nucleic acids such as oligonucleotides and DNA can also be encapsulated in virosomes after condensation with polylysine to form particles that are then enclosed within a virosome for delivery to a cell rather than being complexed to it, thereby minimizing or avoiding, if desired, the use of a cationic lipid. Furthermore, encapsulated DNA is protected from DNase degradation.

Detailed Description Paragraph Right (117):
Additional components may be added to the virosomes to target the virosomes to specific cell types. For example, the virosomes can be conjugated to monoclonal antibodies that

bind to epitopes present only on specific cell types. For example, monoclonal antibodies may bind specifically to cancer-related antigens providing a means for targeting the virosomes following systemic administration. Alternatively, ligands that bind surface receptors of the target cell types may also be bound to the virosomes. Other means for targeting liposomes may also be employed in the present invention.

Detailed Description Paragraph Right (160):
A number of different structures are visible in the micrograph. Much of the lipid is present as large spherical vesicles of 400 to 600 nm in diameter. Many of the vesicles have indentations which appear to be randomly distributed in some vesicles, but organized in straight or curved lines in others. Cusp-like protrusions are also visible on the concave surfaces of some vesicles. These features are commonly referred to as lipidic particles (Verkleij, A. J., Biochim. Biophys. Acta, 779:43-92 (1984)) and may represent an intermediate structure formed during fusion of bilayers. These large vesicles would be expected to give rise to a predominantly bilayer .sup.31 P-NMR spectrum with a narrow isotropic signal due to the lipidic particles. Similar results have been observed with N-methylated PEs (Gagne, et al., Biochemistry, 24:4400-4408 (1985)). A number of smaller vesicles of around 100 nm diameter can also be seen. These vesicles may have been formed spontaneously on hydration, or may have been produced by vesiculization of larger vesicles. These vesicles are sufficiently small for lipid lateral diffusion, or tumbling of the vesicles in suspension, to produce motional averaging on the NMR timescale (Burnell, et al., Biochim. Biophys. Acta, 603:63-69 (1980)), giving rise to an isotropic signal (see, FIG. 2A). In the center of FIG. 6 is a large aggregate showing evidence of several different structures. The right side of the aggregate is characterized by what appears to be closely packed lipidic particles. The upper left hand side shows a distinct organization into three-dimensional cubic arrays and the lower left hand region has the appearance of stacked tubes characteristic of lipid adopting the H.sub..PI. phase (Hope, et al., J. Elect. Micros. Tech., 13:277-287 (1989)). This is consistent with the corresponding .sup.31 P-NMR spectrum.

Detailed Description Paragraph Right (161):
FIG. 7 shows the appearance of the same mixture after extrusion through polycarbonate filters of 100 nm pore size to produce LUVS. The lipid is predominantly organized into vesicles of approximately 100 nm in diameter. Closer inspection reveals the presence of occasional larger vesicles and some of tubular shape. Overall the fairly uniform size distribution is typical of that obtained when liposomes are produced by extrusion.

Detailed Description Paragraph Right (187):
TCS composed of 1,2-dioleoyl-3-phosphatidylethanolamine (DOPE), N,N-dioleoyl-N,N-dimethylammoniumchloride (DODAC), the fluorophores N-(7-nitro-2-1,3-benzoxadiazol-4-yl)-1,2-dioleoyl-sn-phosphatidylethanolam ine (NBD-PE) and N-(lissamine rhodamine B sulfonyl)-1,2-dioleoyl-sn-phosphatidylethanolamine (Rh-PE), and either PEG.sub.2000 -Ceramide (C14:0) or PEG.sub.2000 -DMPE were prepared by extrusion through 100 nm diameter polycarbonate filters (Hope, M. J., et al., P.R. Biochim. Biophys. Acta, 812:55-65 (1985)). TCS contained 0.5 mol % NBD-PE and 0.5 mol % Rh-PE and either DOPE:DODAC:PEG.sub.2000 -DMPE (80:15:5 mol %) or DOPE:DODAC:PEG.sub.2000 -Ceramide (C14:0) (80:15:5 mol %). Fluorescently labelled liposomes were incubated at 37.degree. C. in 20 mM HEPES, 150 mM NaCl, pH 7.4 (HBS) with a three-fold excess of liposomes composed of DOPE:POPS (85:15 mol %). POPC liposomes were added at 10-fold the concentration of the fluorescently labelled liposomes and lipid mixing was assayed by the method of Struck, D. K., et al. (Biochemistry, 20:4093-4099 (1981)). The excitation wavelength used was 465 nm and an emission filter placed at 530 nm minimized intensity due to scattered light. Rates and extents of fusion were followed by monitoring the increase in NBD fluorescence intensity at a wavelength of 535 nm over time. Percent maximum fusion was determined from the relationship Fusion (% max)(t)=(F(t)-F.sub.o)/(F.sub..infin. -F.sub.o), where F.sub.o is the initial NBD fluorescence intensity at time zero, F(t) is the intensity at time t and F.sub..infin. is the maximum achievable fluorescence intensity under conditions of complete lipid mixing of fluorescently labelled and DOPC:POPS liposomes (Bailey, A. L., et al., P.R. Biochemistry, 33:12573-12580 (1994)). FIG. 25 illustrates considerable mixing of DOPE/DODAC/PEG.sub.2000 -Ceramide (C14:0) with DOPC:POPS compared to that of DOPE/DODAC/PEG.sub.2000 -DMPE with DOPC:POPS, suggesting that the PEG.sub.2000 -DMPE is only minimally removed from the TCS. This result is attributed to the electrostatic interaction between the anionic PEG.sub.2000 -DMPE and cationic DODAC which effectively decreases the monomer concentration of the PEG.sub.2000 -DMPE in aqueous solution.

Detailed Description Paragraph Right (196):

Liposomes composed of DOPE/DODAC (85:15, mol/mol), DOPE/DODAC/PEG-Ceriamide (C20) (80:15:5, mol/mol), and DOPE/DODAC/PEG-DSPE (80:15:5, mol/mol) were prepared by the extrusion method and had similar average diameters (100 nm). Freeze-fracture electron micrographs of the three liposomal formulations showed unilamellar liposomes with relatively narrow size ranges. However, preincubation of DOPE/DODAC liposomes in 50% mouse serum at 37.degree. C. for 30 minutes resulted in their massive aggregations. On the other hand, both DOPE/DODAC/PEG-Ceramide (C20) and DOPE/DODAC/PEG-DSPE liposomes did not show any aggregation when these liposomes were pretreated with mouse serum. Thus, these results show the effectiveness of the bilayer stabilizing components in preventing serum-induced rapid aggregations of DOPE/DODAC liposomes.

Detailed Description Paragraph Right (198):
DOPE/DODAC liposomes with or without bilayer stabilizing components were prepared to include .sup.3 H-labelled cholesterolhexadecylether as a lipid marker, and their biodistribution was examined in mice at 1 hour after injection. Liposomes tested in this study were composed of DOPE/DODAC (85:15, mol/mol), DOPE/DODAC/PEG-Ceramide (80:15:5, mol/mol), and DOPE/DODAC/PEG-DSPE (80:15:5, mol/mol). To also examine the effect of the hydrophobic anchor on biodistribution of liposomes, various PEG-Ceramide derivatives with different acyl chain lengths were used. These liposomal formulations had similar average diameters, ranging from 89 to 103 nm. Table II below shows levels of liposomes in the blood, spleen, liver, lung, heart, and kidney, together with respective blood/liver ratios. DOPE/DODAC liposomes were shown to be cleared rapidly from the blood and accumulate predominantly in the liver with the blood/liver ratio of approximately 0.01. Inclusion of bilayer stabilizing components at 5.0 mol % in the lipid composition resulted in their increased blood levels and accordingly decreased liver accumulation to different degrees. DOPE/DODAC/PEG-DSPE liposomes showed the highest blood level (about 59%) and the lowest liver accumulation (about 35%) with the blood/liver ratio of approximately 1.7 at 1 hour after injection. Among various PEG-Ceramide derivatives with different acyl chain lengths, PEG-Ceramide (C20)-containing liposomes showed the highest blood level (about 30%) with the blood/liver ratio of approximately 0.58, while PEG-Ceramide C8)-containing liposomes showed a lower blood level (about 6%) with the blood/liver ratio of approximately 0.1. It appeared that, among different PEG-Ceramide derivatives, the activity in increasing the blood level of liposomes is directly proportional to the acyl chain length of ceramide; the longer the acyl chain length, the greater the activity. It also appeared that the optimal derivative for increasing the blood level of liposomes is PEG-Ceramide (C20).

Detailed Description Paragraph Right (200):
The effect of increasing concentrations of PEG-Ceramide (C20) in the lipid composition on biodistribution of DOPE/DODAC liposomes was examined. PEG-Ceramide (C20) was included in DOPE/DODAC liposomes at increasing concentrations (0-30 mol %) in the lipid composition, while the concentration of DODAC was kept at 15 mol % of the lipid mixture. Liposomes were prepared by the extrusion method and had similar average diameters ranging from 102 nm to 114 nm. Liposomes were injected i.v. into mice, and biodistribution was examined at 1 hour after injections. FIG. 26 shows the liposome level in the blood and liver at 1 hour after injections as a function of the PEG-Ceramide (C20) concentration. Clearly, increasing the concentration of PEG-Ceramide in the lipid composition resulted in progressive increase in liposome levels in the blood, accompanied by decreased accumulation in the liver. The highest blood level (about 84% at 1 hour after injection) was obtained for DOPE/DODAC/PEG-Ceramide (C20) (55:15:30, mol/mol) showing the blood/liver ratio of about 6.5.

Detailed Description Paragraph Right (201):
The effect of increasing concentrations of DODAC on the biodistribution of DOPE/DODAC liposomes also was examined. DOPE/DODAC liposomes containing either 10 mol % or 30 mol % PEG-Ceramide (C20) and various concentrations (15, 30, 50 mol %) were prepared by the extrusion method and had similar average diameters ranging from 103 to 114 nm. Biodistribution was examined at 1 hour after injections, and expressed as percentages of liposomes in the blood as a function of the DODAC concentration (FIG. 27). As shown in FIG. 27, increasing DODAC concentrations in the lipid composition resulted in decreased levels in the blood for both liposomal formulations. Thus, the presence of a cationic lipid, DODAC, in the lipid composition results in rapid clearance from the blood. Also, shown in FIG. 27 is that such a DODAC effect can be counteracted by increasing the concentration of PEG-Ceramide (C20) in the lipid composition.

Detailed Description Paragraph Right (211):
Chloroform solutions of lipids were dried by vortex mixing under nitrogen followed by the removal of residual solvent under high vacuum for 1 hour. When lipopeptide was

incorporated into the liposome preparations, it was added to the dried lipids as a 1 mM solution in DMSO along with an equal volume of benzene-methanol (95:5) prior to freeze-drying for 5 hours. Lipids were hydrated with appropriate buffers to concentrations ranging from 5 to 20 mM lipid. Five freeze-thaw cycles were used to ensure homogeneous mixture of the multilamellar vesicle (MLV) suspensions. The MLVs were extruded 10 times through two 100 nm pore-size polycarbonate filters (Costar, Cambridge, Mass.) to produce large unilamellar vesicles (LUVs). Lipid concentrations were determined by phosphate assay as described previously (see, e.g., Bartlett, G. R., J. Biol. Chem., 234:466-68 (1959)). Depending on the lipid formulation, the mean diameter of the LUVs ranged from 100 to 135 nm as measured by quasi-elastic light scattering.

Detailed Description Paragraph Right (265):
Fusion of EPC/Chol (55:45) LUVs containing 10 mol % Lipo-AcE4K was also observed by freeze-fracture electron microscopy. At pH 5.0, large lipid vesicles with diameters of several hundred nanometers were formed. This limited size increase, compared to the extensive fused structures found in Ca.sup.2+ -induced fusion of negatively charged liposomes, is consistent with the transient destabilization indicated by the lipid mixing and contents mixing results. The freeze-fracture micrographs also show very rough lipid surfaces and extensive cross-fracturing, both of which can be attributed to destabilization of the membrane structure by the lipopeptide. Fluorescence microscopy of erythrocyte ghosts incubated with these liposomes indicated not only pH-dependent lipid mixing with but also aggregation of the ghosts at low pH. No Rh-PE fluorescence was observed in the ghosts at pH 7.5 for either preparation or for EPC/Chol LUVs without Lipo-AcE4K at either pH 7.5 or pH 5.0.

Detailed Description Paragraph Table (2):
TABLE II Effect of Amphipathic PEG Derivatives on Biodistribution of DOPE/DODAC Liposomes PEG- Average % injected dose Derivative Diameter (nm) Blood Liver Spleen Lung Heart Kidney Total Blood/Liver None 103 (29) 0.8 (0.4) 64.4 (2.0) 3.1 (1.8) 1.2 (0.2) 0.2 (0.0) 0.3 (0.0) 70.0 (1.4) 0.012 PEG-DSPE 95 (26) 59.1 (8.2) 34.7 (2.1) 2.9 (0.1) 1.9 (0.8) 1.7 (0.4) 1.2 (0.5) 101.4 (6.1) 1.703 PEG-Cer (C8) 89 (24) 6.5 (1.9) 62.8 (3.4) 4.2 (1.0) 0.5 (0.3) 0.3 (0.1) 0.3 (0.1) 74.6 (5.1) 0.104 PEG-Cer (C14) 93 (25) 5.9 (0.5) 55.9 (1.0) 3.3 (0.2) 0.1 (0.0) 0.1 (0.0) 0.1 (0.0) 65.4 (1.6) 0.106 PEG-Cer (C16) 93 (24) 13.9 (2.1) 57.5 (2.0) 2.6 (0.1) 0.0 (0.0) 0.2 (0.1) 0.0 (0.0) 74.3 (4.0) 0.242 PEG-Cer (C20) 101 (24) 29.8 (4.8) 51.0 (2.2) 1.9 (0.2) 0.0 (0.0) 0.3 (0.1) 0.0 (0.0) 82.8 (2.8) 0.584 PEG-Cer (C24) 92 (28) 26.7 (0.8) 46.7 (7.6) 5.7 (1.2) 1.0 (0.2) 0.9 (0.2) 0.4 (0.1) 81.5 (4.1) 0.572 .sup.3 H-labelled liposomes composed of DOPE/DODAC (75:15, mol/mol) additionally containing an indicated PEG derivative at 5.0 mol % of the lipid mixture were injected i.v. into mice. Biodistribution was examined at 1 h after injection and expressed as percentage of injected dose of liposomes with SD (n = 3).

Other Reference Publication (2):
Saiki et al., "Antimetastatic effects of synthetic polypeptides containing repeated structures of the cell adhesive Arg-Gly-Asp (RGD) and Tyr-IIe-Gly-Ser-Arg (YIGSR) sequences," Br. J. Cancer 60: 722-728 (1989).

Other Reference Publication (3):
Murata et al., "Inhibition of tumour cell adhesion by anti-metastatic polypeptide containing a repetitive Arg-Gly-Asp sequence," Int. J. Biol. Macromol. 11: 226-232 (1989).

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L13: Entry 11 of 38 File: USPT Jul 9, 2002

US-PAT-NO: 6416740
DOCUMENT-IDENTIFIER: US 6416740 B1

TITLE: Acoustically active drug delivery systems

DATE-ISSUED: July 9, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Unger; Evan C.	Tucson	AZ		

US-CL-CURRENT: 424/9.52; 424/450, 424/9.5, 424/9.51

CLAIMS:

What is claimed is:

1. A method for the controlled delivery of a therapeutic compound to a region of a patient comprising: (i) administering to a patient a targeted therapeutic delivery system comprising, in combination with a therapeutic compound, stabilized lipid microspheres encapsulating a gas or gaseous precursor and an oil, wherein said microspheres comprises at least one phosphatidylcholine, at least one phosphatidylethanolamine, and at least one phosphatidic acid, wherein said phosphatidylcholine is selected from the group consisting of dioleoylphosphatidylcholine dimyistoylphosphatidylcholine, dipalmitoylphosphatidylcholine, and distearoyl-phosphatidylcholine; said phosphatidylethanolamine is selected from the group consisting of dipalmitoylphosphatidylethanolamine, dipalmitoylphosphatidylethanolamine-PEG 5,000, dioleoyl-phosphatidylethanolamine, and N-succinyl-dioleoyl-phosphatidylethanolamine; and said phosphatidic acid is dipalmatoylphosphatidic acid; (ii) monitoring the targeted therapeutic delivery system using diagnostic ultrasound to determine the presence of the microspheres in said region; and (iii) applying therapeutic ultrasound to said region to induce rupturing of said microspheres, thereby releasing the therapeutic compound in said region, wherein said therapeutic compound is encapsulated or embedded in said microspheres, and said therapeutic ultrasound is applied at a level below the threshold level for lethal cytotoxicity.

2. A method of claim 1 for use in treating macular degeneration wherein said therapeutic compound comprises a-tocopherol and retinoic acid, said oil is soybean oil, said microspheres comprise 82 mol percent dipalmitoylphosphatidyl choline, 10 mol percent dipalmitoylphosphatidic acid, and 8 mol percent dipalmitoylphosphatidyl ethanolamine-polyethylene glycol 5000, and said gaseous precursor is perfluorobutane.

3. A method of claim 1 for use in treating retinoblastoma wherein said therapeutic compound comprises taxol and retinoic acid, said oil is soybean oil, said microspheres comprise 82 mol percent dipalmitoylphosphatidyl choline, 10 mol percent dipalmitoylphosphatidic acid, and 8 mol percent dipalmitoylphosphatidyl ethanolamine-polyethylene glycol 5000, and said gaseous precursor is perfluorobutane.

4. A method of claim 1 wherein said therapeutic compound is amphotericin-B, said oil is soybean oil, said microspheres comprise 82 mol percent dipalmitoylphosphatidyl choline, 10 mol percent dipalmitoylphosphatidic acid, 8 mol percent dipalmitoylphosphatidylethanolamine-polyethylene glycol 5000 and apoloxamer, and said gaseous precursor is perfluorobutane.

5. A method of claim 4 used to treat fungal ophthalmitis.

6. A method of claim 1 for treating retinitis pigmentosa wherein said therapeutic compound is bendazac, said oil is soybean oil, said microspheres comprise 82 mol percent dipalmitoylphosphatidyl choline, 10 mol percent dipalmitoylphosphatidic acid, 8 mol percent dipalmitoylphosphatidylethanolamine-polyethylene glycol 5000 and a poloxamer, and said gaseous precursor is perfluorobutane.

7. A method of claim 1 for eating benign prostatic hyperplasia wherein said therapeutic compound is doxazosin, said oil is soybean oil, said micropheres comprises 82 mol percent dipalmitoylphosphatidyl choline, 10 mol percent dipalmitoylphosphatidic acid, 8 mol percent dipalmitoylphosphatidyl ethanolamine-polyethylene glycol 5000 and a poloxamner, and said gaseous precursor is perfluorobutane.

8. A method of claim 1 wherein said therapeutic compound is .alpha.-tocopherol, said microspheres comprises CF.sub.3 (CF.sub.2).sub.8 (CH.sub.2).sub.6 COOH, said oil is canola oil, and said gaseous precursor is perfluorobutane.

9. A method of claim 1 wherein said therapeutic compound is a dye, said oil is soybean oil, said micropheres comprises 82 mol percent dipalmitoylphosphatidylcholine, 8 mol percent dipalmitoylphosphatidylethanolamine-polyethylene glycol 5000, and 10 mol percent dipalmitoylphosphatidic acid, and said gaseous precursor is perfluoropropane.

10. A method of claim 1 wherein said therapeutic compound is dexamethasone, said micropheres comprises 82 mol percent dipalmitoylphosphatidylcholine, 8 mol percent dipalmitoylphosphatidylethanolamine-polyethylene glycol 5000, and 10 mol percent dipalmitoylphosphatidic acid, and said gas is perfluorobutane and nitrogen.

11. A method of claim 1 wherein said therapeutic compound is amphotericin, said micropheres comprises 82 mol percent dipalmitoylphosphatidylcholine, 8 mol percent dipalmitoylphosphatidylethanolamine-polyethylene glycol 5000, and 10 mol percent dipalmitoylphosphatidic acid, and said gas is selected from the group consisting of perfluorobutane and nitrogen.

12. A method of claim 1 for treating prostate cancer or benign prostate hypertrophy wherein said therapeutic compound is selected from the group consisting of testosterone, methyltestosterone, fluoxymesterone, finasteride, and Sa reductase enzyme inhibitors.

13. A method according to any preceding claim wherein said diagnostic ultrasound has a frequency equal to 1.times., and wherein said therapeutic ultrasound has a frequency equal to 2.times., 3.times., or 5.times..

14. A method according to claim 13 wherein said therapeutic ultrasound is superimposed upon said diagnostic ultrasound.

15. A method according to claim 14 wherein said therapeutic ultrasound is administered as a train of continuous wave pulses.

WEST

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L13: Entry 36 of 38 File: USPT Aug 18, 1998

DOCUMENT-IDENTIFIER: US 5795587 A
TITLE: Stable lipid-comprising drug delivery complexes and methods for their production

Brief Summary Paragraph Right (2):
The development of new forms of therapeutics which use macromolecules such as proteins or nucleic acids as therapeutic agents has created a need to develop new and effective means of delivering such macromolecules to their appropriate cellular targets. Therapeutics based on either the use of specific polypeptide growth factors or specific genes to replace or supplement absent or defective genes are examples of therapeutics which may require such new delivery systems. Clinical application of such therapies depends not only on efficacy of new delivery systems but also on their safety and on the ease with which the technologies underlying these systems can be adapted for large scale pharmaceutical production, storage, and distribution of the therapeutic formulations. Gene therapy has become an increasingly important mode of treating various genetic disorders. The potential for providing effective treatments, and even cures, has stimulated an intense effort to apply this technology to diseases for which there have been no effective treatments. Recent progress in this area has indicated that gene therapy may have a significant impact not only on the treatment of single gene disorders, but also on other more complex diseases such as cancer. However, a significant obstacle in the attainment of efficient gene therapy has been the difficulty of designing new and effective means of delivering therapeutic nucleic acids to cell targets. Thus, an ideal vehicle for the delivery of exogenous genes into cells and tissues should be highly efficient in nucleic acid delivery, safe to use, easy to produce in large quantity and have sufficient stability to be practicable as a pharmaceutical.

Drawing Description Paragraph Right (7):
FIG. 6 shows the results of CAT assays of tumor extracts prepared from mice having ovarian tumors. 2.times.10.sup.6 human ovarian carcinoma cells were subcutaneously injected into SCID mice at day 0. On day 14, 100 .mu.l solutions containing pUCCMVCAT DNA (contains the chloramphenicol acetyl transferase gene of E. coli) 30 .mu.g) complexed with DC-Chol liposomes (30 nmoles) in the form of admixture (lanes 1 and 2; duplicate samples) or the same amount of DNA in the form of purified complex (prepared from DNA:DC-chol liposome at ratio 1 .mu.g/25 nmoles, lanes 3 and 4; duplicate samples) were directly injected into tumors. 48 hours following transfection, the mice were sacrificed and tumor extract containing 100 .mu.g protein was assayed for CAT activity. Lane 5 shows positive control CAT activity for standard E. coli CAT.

Detailed Description Paragraph Right (16):
The cationic liposomes mixed with drug or with drug and polycation to form the complexes of the present invention may contain a cationic lipid alone or a cationic lipid in combination with a neutral phospholipid. Suitable cationic lipid species include, but are not limited to: 1,2bis(oleoyloxy)-3-(trimethylammonio) propane (DOTAP); N-[1,-(2,3-dioleoyloxy)propyl]-N,N,N-trimethyl ammonium chloride (DOTMA) or other N-(N, N-1-dialkoxy)-alklyl-N,N,N-trisubstituted ammonium surfactants; 1,2 dioleoyl-3-(4'-trimethylammonio) butanoyl-sn-glycerol (DOBT) or cholesteryl (4' trimethylammonia) butanoate (ChOTB) where the trimethylammonium group is connected via a butanoyl spacer arm to either the double chain (for DOTB) or cholesteryl group (for ChOTB); DORI (DL-1,2-dioleoyl-3-dimethylaminopropyl-B-hydroxyethylammonium) or DORIE (DL-1,2-O-dioleoyl-3-dimethylaminopropyl-.beta.-hydroxyethylammonium) (DORIE) or analogs thereof as disclosed in WO 93/03709; 1,2-dioleoyl-3-succinyl-sn-glycerol choline ester (DOSC); cholesteryl hemisuccinate ester (ChOSC); lipopolyamines such as doctadecylamidoglycylspermine (DOGS) and dipalmitoyl phosphatidyesthanolamidospermine (DPPES) or the cationic lipids disclosed in U.S. Pat. No. 5,283,185, cholesteryl-3.beta.-carboxyl-amido-ethylenetrimethylammonium iodide,

1-dimethylamino-3-trimethylammonio-DL-2-propylcholesteryl carboxylate iodide, cholesteryl-3.beta.-carboxyamidoethyleneamine, cholesteryl-3.beta.-oxysuccinamido-ethylenetrimethylammonium iodide, 1-dimethylamino-3-trimethylammonio-DL-2-propyl-cholesteryl-3.beta.-oxysucc inate iodide, 2-[(2-trimethylammonio)-ethylmethylamino]ethyl-cholesteryl-3.beta.-oxysucc inate iodide, 3.beta.[N-(N',N'-dimethylaminoethane)carbamoyl]cholesterol (DC-chol), and 3.beta.-[N-(polyethyleneimine)-carbamoyl]cholesterol.

Detailed Description Paragraph Right (22):
Methods for producing the liposomes to be used as in the product of the lipid-comprising drug delivery complexes of starting components the present invention are known to those of ordinary skill in the art. A review of methodologies of liposome preparation may be found in Liposome Technology (CFC Press NY 1984); Liposomes by Ortro (Marcel Schher, 1987); Methods Biochem Anol. 33:337-462 (1988) and U.S. Pat. No. 5,283,185. Such methods include freeze-thaw extrusion and sonication. Both unilamellar liposomes (less than about 200 nm in average diameter) and multilamellar liposomes (greater than about 300 nm in average diameter) may be used as starting components to produce the complexes of this invention.

Detailed Description Paragraph Right (23):
In the cationic liposomes utilized to produce the drug/lipid complexes of this invention, the cationic lipid is present in the liposome at from about 10 to about 100 mole % of total liposomal lipid, preferably from about 20 to about 80 mole % and most preferably about 20 to about 60 mole %. The neutral lipid, when included in the liposome, may be present at a concentration of from about 0 to about 90 mole % of the total liposomal lipid, preferably from about 20 to about 80 mole %, and most preferably from 40 to 80 mole %. The negatively charged lipid, when included in the liposome, may be present at a concentration ranging from about 0 mole % to about 49 mole % of the total liposomal lipid, preferably from about 0 mole % to about 40 mole %. In a preferred embodiment, the liposomes contain a cationic and a neutral lipid, most preferably DC-Chol and DOPE in ratios between about 2:8 to about 6:4. It is further understood that the complexes of the present invention may contain modified lipids, protein, polycations or receptor ligands which function as a targeting factor directing the complex to a particular tissue or cell type. Examples of targeting factors include, but are not limited to, asialoglycoprotein, insulin, low density lipoprotein (LDL), folate and monoclonal and polyclonal antibodies directed against cell surface molecules. Potential targets include, but are not limited to, liver, blood cells, endothelial cells and tumor cells.

Detailed Description Paragraph Right (29):
The diameter of the complexes produced by the methods of the present invention is less than about 400 nm, preferably less than about 200 nm, and more preferably less than 150 nm.

Detailed Description Paragraph Right (36):
Cationic liposomes at a 20 mM total lipid concentration were prepared from DC-Chol and DOPE at various ratios by a sonication method, according to a published procedure (Gao, X., and Huang, L. (1991) Biochem. Biophys. Res. Commun., 179: 280-285). Trace amount of [.sup.3 H] cholesteryl hexadecyl ether (Amersham, Arlington Heights, Ill.) was included for quantitation purpose. The size of these liposomes was between 100 to 150 nm in diameter, as determined by quasi-elastic light scattering using a Coulter N4SD particle sizer (Coulter Electronics, Inc., Hialeah, Fla.). Unless indicated otherwise in the following Examples, DNA/lipid complexes were prepared at a typical laboratory scale by adding amounts of free DC-Chol/DOPE liposomes as indicated in each Example in a volume of 1 ml of a 2 mM Hepes buffer (pH 7.6) to a 15.times.7.5 polystyrene culture tube (Baxter, McGraw Pare, Ill.), a micro-magnetic stirrer was placed in the tube, and the solution was well mixed. Amounts of pRSVL DNA as indicated in each Example were then added dropwise from a stock solution (0.2 mg/ml, in 2 mM Hepes buffer, pH 7.6) to the liposome solution over a period of 3 min. Trace amounts of pRSVL labeled with 32P using a nick translation kit (Promega, Madison, Wis.) and [32P] dCTP (Amersham, Arlington Heights, Ill.) was included for the purpose of quantitation. To prepare lipid/PLL/DNA complexes, an amount of the above 0.2 mg/ml DNA solution as indicated in each Example was added to 1 ml PLL/liposome mixture containing amounts of liposomes and PLL as indicated in each Example. DNA/lipid complexes were loaded on the top of a sucrose step gradient composed of 0.5 ml each of 5%, 7.5%, 10% and 15% sucrose (w/w) and DNA/lipid/PLL complexes were loaded on top of a sucrose step gradient composed of 0.5 ml each of 5%, 10%, 15%, 20%, 25% and 30% sucrose (w/w). The DNA/lipid and DNA/lipid/PLL complexes were then purified from free lipid and PLL by ultracentrifugation at 100,000 g for 30 min at 4.degree. C. After centrifugation,

fractions of 200 .mu.l were taken from the top to the bottom of the tube. Aliquots from each fraction were assayed for both .sup.3 H and .sup.32 P radioactivity using a scintillation counter. Fractions that contained peak value of the 32P were collected and pooled. These pooled fractions were then assayed for particle size and for transfection activity.

Detailed Description Paragraph Right (53):
3.times.10.sup.6 human ovarian carcinoma cells were injected subcutaneously into SCID mice at day 0. 14 days later, 100 .mu.l solutions containing pUCCMVCAT DNA (30 .mu.g) complexed with DC-Chol (3:2 DC-Chol:DOPE) liposomes (30 nmoles) in the form of admixture (lanes 1 and 2) or the same amount of DNA in the form of purified DNA/lipid complex (prepared from DNA and DC-Chol liposomes at ratios of 1 .mu.g DNA/25 nmoles lipid were directly injected into tumors. Animals were sacrificed 2 days later and tumor extracts containing 100 .mu.g protein were assayed for CAT activity at 37.degree. C. according to Ausubel, et al. (1991) Current Protocols in Molecular Biology (Wiley, Boston), Vol. 1, pp. 9.6.2-9.6.5). The results show that purified complex, while prepared under non-optimal conditions, exhibited in vivo transfection activity.

Detailed Description Paragraph Center (16):
In Vivo Transfection of Tumors by Purified DNA/Lipid Complexes

CLAIMS:

1. A method for producing noncovalent nucleic acid/lipid/polycation complexes having a net positive charge at about pH 6.0-8.0, said method comprising mixing said nucleic acid with cationic liposomes and polycation in a ratio of nucleic acid to lipid to polycation which results in the production of said complexes, said polycation being selected from the group consisting of polyarginine, polyornithine, protamines, polylysine, polybrene (hexadimethrine bromide), histone, cationic dendrimer, spermine, spermidine and synthetic polypeptides derived from SV40 large T antigen, and which synthetic polypeptides have excess positive charges and represent a nuclear localization signal.

7. The method of claim 1, wherein said complex has an average diameter less than about 400 nm.

8. A noncovalent nucleic acid lipid/polycation complex having a net positive charge at pH 6.0-8.0; said complex being produced by mixing cationic liposomes with polycation and nucleic acid in a ratio of nucleic acid to lipid to polycation which results in the production of said complex, said polycation being selected from the group consisting of polyarginine, polyornithine, protamines, polylysine, polybrene (hexadimethrine bromide), histone, cationic dendrimer, spermine, spermidine and synthetic polypeptides derived from SV40 large T antigen and which synthetic polypeptides have excess positive charges and represent a nuclear localization signal.

(FILE 'HOME' ENTERED AT 11:11:26 ON 10 JUL 2002)

FILE 'MEDLINE, CANCERLIT, BIOTECHDS, BIOSIS, EMBASE, CAPLUS' ENTERED AT 11:12:24 ON 10 JUL 2002

L1	777021	S LIPOSOME OR CATIONIC LIPID OR LIPID OR AMPHIPHILE
L2	807868	S NM OR NANOMET?
L3	20252	S L2 AND L1
L4	307698	S DIAMETER
L5	2606	S L4 AND L3
L6	272350	S (HUNDRE? OR 200 OR 100 OR 150 OR 50) AND L2
L7	1185	S L6 AND L5
L8	465	S MEAN AND L5
L9	220	DUP REM L8 (245 DUPLICATES REMOVED)
L10	4589200	S DNA OR NUCLEIC OR PLASMID OR POLYNUCLEOTIDE OR GENE
L11	20	S L10 AND L9
L12	117	S L5 AND CATIONIC
L13	53	DUP REM L12 (64 DUPLICATES REMOVED)
L14	518416	S LIGAND OR (TRANSFERIN OR FOLATE)
L15	1	S L14 AND L13
L16	41	S L14 AND L5
L17	18	DUP REM L16 (23 DUPLICATES REMOVED)
L18	3570293	S TUMOR OR CANCER
L19	46117	S L18 AND L1
L20	1015	S L19 AND L14
L21	419	S L20 AND L10
L22	247	DUP REM L21 (172 DUPLICATES REMOVED)
L23	34	S L22 AND CATIONIC

L11 ANSWER 13 OF 20 BIOTECHDS

AN 1995-04415 BIOTECHDS

TI The effect of lipofectin complexation on the characteristics and stability of **DNA** expression vectors;
cationic **liposome**-mediated lipofection; **DNA** expression vector **plasmid** stability (conference abstract)

AU Gong L; Claspell J; Rolland A

CS GeneMedicine

LO GeneMedicine, Inc., Houston, TX 77054, USA.

SO Pharm.Res.; (1994) 11, 10, Suppl., S77
CODEN: PHREEB ISSN: 0724-8741
AAPS 94, San Diego, California, 6-10 November, 1994.

DT Journal

LA English

AB Cationic **liposome**-medicated transfection allows efficient delivery of **DNA** into a wide variety of eukaryotic cells and to result in relatively high levels of expression of the exogenous genes. Lipofectin is a positively charged **liposome** preparation composed of an equimolar mixture of DOTMA (N-(1-(2,3-dioleoyloxy)propyl)-N,N,N-trimethylammonium chloride) and DOPE (dioleoyl phosphatidylethanolamine). **DNA**-lipofection complexes of different ratios were characterized by laser light scattering (hydrodynamic size morphology), Doppler electrophoresis and TEM. The effect of complexation on the degradation of expression vectors were studied using either mechanical (ultrasonication) or enzymatic (**DNA**-ase-1) method. The results show that the **DNA** -lipofectin complexes are present in monodisperse size distribution with a **mean diameter** of about 200 **nm**. Zeta potential of the complex is dependent on the **DNA**-lipofectin ratio. The stability of the expression vector increased significantly after forming complexes with lipofectin. The protective effect also depends on **DNA**-lipofectin ratio. (0 ref)

L11 ANSWER 10 OF 20 MEDLINE
AN 96215256 MEDLINE
DN 96215256 PubMed ID: 8626549
TI Folate-targeted, anionic **liposome**-entrapped polylysine-condensed **DNA** for tumor cell-specific **gene** transfer.
AU Lee R J; Huang L
CS Department of Pharmacology, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania 15261, USA.
NC CA59327 (NCI)
DK44935 (NIDDK)
HL50256 (NHLBI)
\+
SO JOURNAL OF BIOLOGICAL CHEMISTRY, (1996 Apr 5) 271 (14) 8481-7.
Journal code: 2985121R. ISSN: 0021-9258.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199606
ED Entered STN: 19960708
Last Updated on STN: 19970203
Entered Medline: 19960621
AB We have developed a lipidic **gene** transfer vector, LPDII, where **DNA** was first complexed to polylysine at a ratio of 1:0.75 (w/w) and then entrapped into folate-targeted pH-sensitive anionic liposomes composed of dioleoyl phosphatidylethanolamine (DOPE)/cholesteryl hemisuccinate/folate-polyethlene glycol-DOPE (6:4:0.01 mol/mol) via charge interaction. LPDII transfection of KB cells, a cell line overexpressing the tumor marker folate receptor, was affected by both the **lipid** to **DNA** ratio and the **lipid** composition. At low **lipid** to **DNA** ratios (e.g. 4 and 6), LPDII particles were positively charged; transfection and cellular uptake levels were independent of the folate receptor and did not require a pH-sensitive **lipid** composition. Meanwhile, transfection and uptake of negatively charged LPDII particles, i.e. those with high **lipid** to **DNA** ratios (e.g. 10 and 12), were folate receptor-dependent and required a pH-sensitive **lipid** composition. The transfection activity of LPDII was lost when the inverted cone-shaped DOPE was replaced by dioleoyl phosphatidylcholine. LPDII particles with **lipid** to **DNA** ratios of 4, 6, 10, and 12 were approximately 20-30 times more active than **DNA**.3-beta-[N-(N',N'-dimethylethane)carbamoyl]choles terol cationic **liposome** complexes in KB cells and were much less cytotoxic. On the sucrose gradient, LPDII particles had a migration rate in between those of the free **DNA** and the **DNA** .polylysine complex. An electron micrograph of LPDII showed a structure of spherical particles with a positively stained core enclosed in a lipidic envelope with a **mean diameter** of 74 +/- 14 **nm** . This novel **gene** transfer vector may potentially be useful in **gene** therapy for tumor-specific delivery.

L11 ANSWER 9 OF 20 MEDLINE
AN 97246097 MEDLINE
DN 97246097 PubMed ID: 9090708
TI Self-assembling **DNA-lipid** particles for **gene** transfer.
AU Zhang Y P; Reimer D L; Zhang G; Lee P H; Bally M B
CS British Columbia Cancer Agency, Division of Medical Oncology, Vancouver, Canada.
SO PHARMACEUTICAL RESEARCH, (1997 Feb) 14 (2) 190-6.
Journal code: 8406521. ISSN: 0724-8741.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199706
ED Entered STN: 19970630
Last Updated on STN: 19970630
Entered Medline: 19970617

AB PURPOSE: We have demonstrated that a heteromolecular complex consisting of cationic lipids and **DNA** can be prepared and isolated (1). Cationic lipids bind **DNA** through electrostatic interactions. However, when sufficient lipids are bound to **DNA** the physical and chemical properties of the complex are governed by hydrophobic effects. Here we describe an approach where this hydrophobic complex is used as an intermediate in the preparation of **lipid-DNA** particles (LDPs). METHODS: The approach relies on the generation of mixed micelles containing the detergent, n-octyl beta-D-glucopyranoside (OGP), the **cationic lipid**, N-N-dioleoyl-N, N-dimethylammonium chloride (DODAC), and selected zwitterionic lipids, 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE) or egg sphingomyelin (SM). RESULTS: When these micelles were prepared at low detergent concentrations (20 mM OGP) and combined with pCMV beta **DNA**, LDPs spontaneously formed. The **mean diameter** of these particles as measured by quasielastic light scattering was 55-70 **nm**, a result that was confirmed by negative stain electron microscopy. Further characterization of these LDPs showed that **DNA** within the particles was inaccessible to the small fluorochrome TO-PRO-1 and protected against DNase I degradation. LDPs could also be prepared in high concentrations of OGP (100 mM), however particles formed only after removal of OGP by dialysis. Particles formed in this manner were large (> 2000 **nm**) and mediated efficient transfection of Chinese hamster ovary cells. Transfection activity was greater when the **lipid** composition used consisted of SM/ DODAC. Small particles (< 100 **nm**) prepared of SM/DODAC were, however, inefficient transfecting agents. CONCLUSIONS: We believe that LDP formation is a consequence of the molecular forces that promote optimal hydrocarbon-hydrocarbon interactions and elimination of the hydrocarbon-water interface.

L11 ANSWER 8 OF 20 MEDLINE
AN 1998070765 MEDLINE
DN 98070765 PubMed ID: 9405626
TI Virus-sized self-assembling lamellar complexes between **plasmid DNA** and cationic micelles promote **gene** transfer.
AU Pitard B; Aguerre O; Airiau M; Lachages A M; Boukhnikachvili T; Byk G; Dubertret C; Herviou C; Scherman D; Mayaux J F; Crouzet J
CS Vector Development, Rhone-Poulenc Rorer Gencell, 13 quai Jules Guesde, BP 14, 94403 Vitry-sur-seine Cedex, France.. bruno.pitard@rp-rorer.fr
SO PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1997 Dec 23) 94 (26) 14412-7.
Journal code: 7505876. ISSN: 0027-8424.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199802
ED Entered STN: 19980217
Last Updated on STN: 19980217
Entered Medline: 19980202
AB **Gene** therapy is based on the vectorization of genes to target cells and their subsequent expression. Cationic **amphiphile** -mediated delivery of **plasmid DNA** is the nonviral **gene** transfer method most often used. We examined the supramolecular structure of lipopolyamine/**plasmid DNA** complexes under various condensing conditions. **Plasmid DNA** complexation with lipopolyamine micelles whose **mean diameter** was 5 **nm** revealed three domains, depending on the lipopolyamine/**plasmid DNA** ratio. These domains respectively corresponded to negatively, neutrally, and positively charged complexes. Transmission electron microscopy and x-ray scattering experiments on complexes originating from these three domains showed that although their morphology depends on the lipopolyamine/**plasmid DNA** ratio, their particle structure consists of ordered domains characterized by even spacing of 80 A, irrespective of the **lipid** /**DNA** ratio. The most active lipopolyamine/**DNA** complexes for **gene** transfer were positively charged. They were characterized by fully condensed **DNA** inside spherical particles (**diameter**: 50 **nm**) sandwiched between **lipid** bilayers. These results show that supercoiled **plasmid DNA** is able to transform lipopolyamine micelles into a supramolecular organization characterized by ordered lamellar domains.

L11 ANSWER 7 OF 20 MEDLINE
AN 1998276742 MEDLINE
DN 98276742 PubMed ID: 9614558
TI Time-dependent maturation of cationic **liposome-DNA** complex for serum resistance.
AU Yang J P; Huang L
CS Department of Pharmacology, University of Pittsburgh School of Medicine, PA 15261, USA.
NC CA64654 (NCI)
CA71731 (NCI)
DK44935 (NIDDK)
SO GENE THERAPY, (1998 Mar) 5 (3) 380-7.
Journal code: 9421525. ISSN: 0969-7128.
CY ENGLAND: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199806
ED Entered STN: 19980708
Last Updated on STN: 19980708
Entered Medline: 19980622
AB Following our previous finding that increasing charge ratio (+/-) can overcome the serum sensitivity of cationic **liposome-DNA** complex, in the present studies we report that serum sensitivity of cationic **liposome-DNA** complex could also be overcome by prolonging the incubation time of the complex. Such time-dependent maturation of the complex was **cationic lipid** -dependent; complexes containing monovalent cationic lipids, eg DC-chol, DOTAP and lipofectin, matured with time, but those containing multivalent **lipid**, eg lipofectamine, do not. High charge ratio high concentration and high temperature accelerated the process of maturation. The serum sensitivity of cellular uptake of **DNA** was also dependent on the incubation time. The matured cationic **liposome-DNA** complexes are homogeneous particles with a **mean diameter** of 170 to 400 **nm**, depending on the **cationic lipid** in the complex.

L11 ANSWER 3 OF 20 MEDLINE
AN 2000161136 MEDLINE
DN 20161136 PubMed ID: 10694807
TI Glomerular filtration is required for transfection of proximal tubular cells in the rat kidney following injection of **DNA** complexes into the renal artery.
AU Foglieni C; Bragonzi A; Cortese M; Cantu L; Boletta A; Chiossone I; Soria M R; Monaco L
CS Biotechnology Unit, Dibit, Department of Biological and Technological Research, San Raffaele Scientific Institute, Milan, Italy.
SO GENE THERAPY, (2000 Feb) 7 (4) 279-85.
Journal code: 9421525. ISSN: 0969-7128.
CY ENGLAND: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200003
ED Entered STN: 20000327
Last Updated on STN: 20000327
Entered Medline: 20000316
AB **Gene** transfer to the kidney can be achieved with various **DNA** vectors, resulting in transgene expression in glomerular or tubular districts. Controlling transgene destination is desirable for targeting defined renal cells for specific therapeutic purposes. We previously showed that injection of polyplexes into the rat renal artery resulted in transfection of proximal tubular cells. To investigate whether this process involves glomerular filtration of the **DNA** -containing particles, fluorescent polyethylenimine polyplexes were prepared, containing fluoresceinated poly-L-lysine. This allowed visualization of the route of the particles into the kidney. Our polyplexes were filtered through the glomerulus, since fluorescent proximal tubuli were observed. Conversely, fluorescent lipopolyplexes containing the **cationic lipid** DOTAP were never observed in tubular cells. Size measurements by laser light scattering showed that the **mean diameter** of polyplexes (93 **nm**) was smaller than that of lipopolyplexes (160 **nm**). The size of the transfecting particles is therefore a key parameter in this process, as expected by the constraints imposed by the glomerular filtration barrier. This information is relevant, in view of modulating the physico-chemical properties of **DNA** complexes for optimal transgene expression in tubular cells. **Gene** Therapy (2000) 7, 279-285.

L11 ANSWER 2 OF 20 MEDLINE
AN 2000228320 MEDLINE
DN 20228320 PubMed ID: 10765503
TI Formulations which increase the size of lipoplexes prevent serum-associated inhibition of transfection.
AU Turek J; Dubertret C; Jaslin G; Antonakis K; Scherman D; Pitard B
CS UMR7001, Vectorologie Moleculaire et Cellulaire, Ecole Nationale Superieure de Chimie de Paris, France.
SO JOURNAL OF GENE MEDICINE, (2000 Jan-Feb) 2 (1) 32-40.
Journal code: 9815764. ISSN: 1099-498X.
CY ENGLAND: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200006
ED Entered STN: 20000629
Last Updated on STN: 20000629
Entered Medline: 20000620
AB BACKGROUND: Cationic lipids are the most widely used nonviral vectors for **gene** delivery. Upon complexation to **DNA,** they offer a nonimmunogenic alternative to viral **gene** transfer. Unfortunately, their in vivo application has been limited due to a serum-associated inhibition of transfection. As a result, significant research effort has focused on overcoming this deleterious effect of serum. METHODS: To better understand this phenomenon, we investigated the influence of lipoplex colloidal stability on **gene** transfection in the presence of serum. In addition, conditions of the reaction medium were modulated and their effects on collidal stability and subsequent in vitro transfection efficiency were studied. RESULTS: The colloidal stability of the **cationic lipid-DNA** complexes, which depended on the charge ratio, determined the efficiency of in vitro transfection in the presence of serum. In particular, large-sized, colloidally unstable complexes of over 700 **nm** **mean diameter** induced efficient transfection in the presence or absence of serum. Conversely, colloidally stable complexes of less than 250 **nm** in size resulted in efficient transfection only in the absence of serum. Furthermore, for the same charge ratio, both colloidally stable and unstable lipoplexes could be obtained depending on the degree to which various solution parameters (NaCl concentration, **cationic lipid** acyl chain length, pH and **DNA** concentration) were altered. In each case, only those complexes lacking colloidal stability resulted in high levels of in vitro transfection in the presence of serum. This phenomenon was shown to be independent of both the percent **DNA** internalized and of the lamellar organization of the **cationic lipid/DNA** lipoplexes.
CONCLUSIONS: Through the modulation of various mixture conditions, large-sized lipoplexes can be formed which are resistant to the transfection-inhibiting effect of serum.

L15 ANSWER 1 OF 1 MEDLINE
AN 96215256 MEDLINE
DN 96215256 PubMed ID: 8626549
TI **Folate**-targeted, anionic **liposome**-entrapped
polylysine-condensed DNA for tumor cell-specific gene transfer.
AU Lee R J; Huang L
CS Department of Pharmacology, University of Pittsburgh School of Medicine,
Pittsburgh, Pennsylvania 15261, USA.
NC CA59327 (NCI)
DK44935 (NIDDK)
HL50256 (NHLBI)
+
SO JOURNAL OF BIOLOGICAL CHEMISTRY, (1996 Apr 5) 271 (14) 8481-7.
Journal code: 2985121R. ISSN: 0021-9258.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199606
ED Entered STN: 19960708
Last Updated on STN: 19970203
Entered Medline: 19960621
AB We have developed a lipidic gene transfer vector, LPDII, where DNA was first complexed to polylysine at a ratio of 1:0.75 (w/w) and then entrapped into **folate**-targeted pH-sensitive anionic liposomes composed of dioleoyl phosphatidylethanolamine (DOPE)/cholesteryl hemisuccinate/**folate**-polyethlene glycol-DOPE (6:4:0.01 mol/mol) via charge interaction. LPDII transfection of KB cells, a cell line overexpressing the tumor marker **folate** receptor, was affected by both the **lipid** to DNA ratio and the **lipid** composition.
At low **lipid** to DNA ratios (e.g. 4 and 6), LPDII particles were positively charged; transfection and cellular uptake levels were independent of the **folate** receptor and did not require a pH-sensitive **lipid** composition. Meanwhile, transfection and uptake of negatively charged LPDII particles, i.e. those with high **lipid** to DNA ratios (e.g. 10 and 12), were **folate** receptor-dependent and required a pH-sensitive **lipid** composition. The transfection activity of LPDII was lost when the inverted cone-shaped DOPE was replaced by dioleoyl phosphatidylcholine. LPDII particles with **lipid** to DNA ratios of 4, 6, 10, and 12 were approximately 20-30 times more active than DNA.3-beta-[N-(N',N'-dimethylethane)carbamoyl]cholesterol **cationic liposome** complexes in KB cells and were much less cytotoxic. On the sucrose gradient, LPDII particles had a migration rate in between those of the free DNA and the DNA.polylysine complex. An electron micrograph of LPDII showed a structure of spherical particles with a positively stained core enclosed in a lipidic envelope with a mean **diameter** of 74 +/- 14 **nm**. This novel gene transfer vector may potentially be useful in gene therapy for tumor-specific delivery.

AN 1993-00840 BIOTECHDS

TI Aerosol gene delivery in vivo;
chloramphenicol-acetyltransferase reporter gene complexation to **cationic liposome** for lipofection of mouse lung;
potential application to human gene therapy

AU Stribling R; Brunette E; Liggitt D; Gaensler K; *Debs R
LO Cancer Research Institute, University of California, San Francisco, CA 94143-0128, USA.

SO Proc.Natl.Acad.Sci.U.S.A.; (1992) 89, 23, 11277-81
CODEN: PNASA6
DT Journal
LA English

AB Vector plasmid pCIS-CAT containing the bacterial chloramphenicol-acetyltransferase (CAT, EC-2.3.1.28) reporter gene fused to the human cytomegalo virus immediate early promoter-enhancer element was complexed with liposomes (100 **nm diameter**) containing the **cationic lipid** DOTMA as DOTMA/DOPE (1:1 mole ratio).
Mice were exposed to an aerosol generated from a solution containing 12 mg of the plasmid alone or to an aerosol generated from a solution containing 12 mg of plasmid complexed to 24 umol of liposomes. Aerosols were administered to animals placed in nose-out cones in an Intox small animal exposure chamber. Significant CAT gene expression was seen only in mice exposed to aerosolized DNA/**liposome** complexes. High levels of CAT activity were present in the lungs for at least 21 days following a single aerosol dose, and no CAT activity was detectable in heart, spleen, kidneys or liver. Immunostaining for intracellular CAT showed that most of airway epithelial and alveolar lining cells were transfected in vivo. There was no apparent treatment-related damage. The results have implications for human gene therapy. (32 ref)

L13 ANSWER 43 OF 53 MEDLINE DUPLICATE 25
AN 94132017 MEDLINE
DN 94132017 PubMed ID: 8300583
TI Enhanced gene delivery and mechanism studies with a novel series of **cationic lipid** formulations.
AU Felgner J H; Kumar R; Sridhar C N; Wheeler C J; Tsai Y J; Border R; Ramsey P; Martin M; Felgner P L
CS VICAL Inc., San Diego, California 92121.
SO JOURNAL OF BIOLOGICAL CHEMISTRY, (1994 Jan 28) 269 (4) 2550-61.
Journal code: 2985121R. ISSN: 0021-9258.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199403
ED Entered STN: 19940318
Last Updated on STN: 19940318
Entered Medline: 19940304
AB The application of **cationic liposome** reagents has advanced DNA and mRNA transfection research in vitro, and data are accumulating which show their utility for in vivo gene transfer. However, chemical structure-activity data leading to a better mechanistic understanding of their biological activity is still limited. Most of the **cationic lipid** reagents in use today for this application are formulated as liposomes containing two **lipid** species, a **cationic amphiphile** and a neutral phospholipid, typically dioleoylphosphatidylethanolamine (DOPE). The studies reported here examine the effects of some systematic chemical structural changes in both of these **lipid** components.
Cationic and neutral phospholipids were formulated together as large multilamellar vesicles (MLV) or small sonicated unilamellar vesicles (SUV) in water, and each formulation was assayed quantitatively in 96-well microtiter plates under 64 different assay conditions using COS.7 cells and an RSV-beta-galactosidase expression plasmid. The **cationic lipid** molecules used for these studies were derived from a novel series of 2,3-dialkyloxypropyl quaternary ammonium compounds containing a hydroxyalkyl moiety on the quaternary amine. A homologous series of dioleylalkyl (C18:1) compounds containing increasing hydroxyalkyl chain lengths on the quaternary amine were synthesized, formulated with 50 mol % DOPE, and assayed for transfection activity. The order of efficacy was ethyl > propyl > butyl > pentyl > 2,3-dioleyloxypropyl-1-trimethyl ammonium bromide (DOTMA). DOTMA, which is commercially available under the trademark Lipofectin Reagent, lacks a hydroxyalkyl moiety on the quaternary amine. A homologous series of hydroxyethyl quaternary ammonium derivatives with different alkyl chain substitutions were synthesized, formulated with 50 mol % DOPE, and assayed in the transfection assay. The order of transfection efficacy was dimyristyl (di-C14:0) > dioleyl (di-C18:1) > dipalmityl (di-C16:0) > disteryl (di-C18:0). The addition of 100 microM chloroquine in the transfection experiment enhanced the activity of the dioleyl compound by 4-fold and decreased the activity of the dimyristyl compound by 70%. For each of the compounds and formulations examined in this report, large multilamellar vesicles (MLV; **diameter** 300-700 **nm**) were more active than small unilamellar vesicles (SUV; **diameter** 50-100 **nm**). The neutral phospholipid requirements for transfection activity in COS.7 cells with these **cationic lipid** molecules were examined.(ABSTRACT TRUNCATED AT 400 WORDS)

L13 ANSWER 41 OF 53 MEDLINE DUPLICATE 24

AN 96146439 MEDLINE

DN 96146439 PubMed ID: 8547238

TI Potentiation of **cationic liposome**-mediated gene delivery by polycations.

AU Gao X; Huang L

CS Department of Pharmacology, University of Pittsburgh School of Medicine, Pennsylvania 15261, USA.

NC CA 59327 (NCI)
DK 44935 (NIDDK)
HL 50256 (NHLBI)

SO BIOCHEMISTRY, (1996 Jan 23) 35 (3) 1027-36.
Journal code: 0370623. ISSN: 0006-2960.

CY United States

DT Journal; Article; (JOURNAL ARTICLE)

LA English

FS Priority Journals

EM 199602

ED Entered STN: 19960306
Last Updated on STN: 19970203
Entered Medline: 19960222

AB We discovered that several high molecular weight **cationic** polymers, such as poly(L-lysine) and protamine, can enhance the transfection efficiency of several types of **cationic** liposomes by 2-28-fold in a number of cell lines in vitro. Small polycations such as spermine and a **cationic** decapeptide derived from SV40 T-antigen were only moderately active. The addition of poly(L-lysine) and protamine dramatically reduced the particle size of the complex formed between DNA and **cationic** liposomes and rendered DNA resistant to the nuclease activity. The complexes composed of DNA, poly(L-lysine), and **cationic** lipids were purified from an excess of free liposomes with sucrose gradient ultracentrifugation. Purified complex formed at low **cationic liposome** ratio was poor in **lipid** content and only had weak transfection activity. Addition of free **liposome** to the purified complex significantly enhanced the transfection activity. In contrast, complexes formed at a higher initial ratio of **liposome** to DNA had a higher **lipid** content and were highly active in transfection; the activity was about 3-9-fold more active than the corresponding complex before purification. Negative stain EM studies revealed that the most active complexes prepared from 40 nmol of **lipid**, 0.5 micrograms of poly(L-lysine), and 1 microgram of DNA and purified by gradient ultracentrifugation were spherical, electron dense, small (< 100 **nm** in **diameter**) particles, and some of them were associated with **lipid** membranes. These highly active, stable, small-sized **lipid** /poly(L-lysine)/DNA complexes represent a new class of nonviral gene delivery vehicles that might be useful in gene therapy.

L13 ANSWER 38 OF 53 BIOTECHDS COPYRIGHT 2002 THOMSON DERWENT AND ISI
AN 1997-13269 BIOTECHDS
TI Novel supramolecular assemblies for gene transfer;
lipid-entrapped polycation-condensed DNA composition for use in lipofection and gene therapy (conference abstract)
AU Huang L
CS Univ.Pittsburgh
LO University of Pittsburgh, Department of Pharmacology, W1351 Biomedical Science Tower, Pittsburgh, PA 15261, USA.
SO Abstr.Pap.Am.Chem.Soc.; (1997) 213 Meet., Pt.2, PMSE306
CODEN: ACSRAL ISSN: 0065-7727
American Chemical Society, 213th ACS National Meeting, San Francisco, CA, 13-17 April, 1997.
DT Journal
LA English
AB The relatively non-toxic and efficient **cationic** **liposome** formulation DC-Chol-DOPE has been used in 2 separate clinical trials for immunotherapy of cancer and gene therapy of cystic fibrosis. Two types of novel condensed structure containing DNA polycation and lipids have been developed. These **lipid** -entrapped polycation-condensed DNA (LPD) particles are small (under 100 **nm** in **diameter**), monodispersed and colloidally stable. Transfection activity of LPD is similar to that of adeno virus vectors, and is 10- to 100-fold higher than that of first-generation **cationic** liposomes. LPD-I particles are **cationic** and used primarily in local and regional delivery routes. LPD-II particles are anionic and may be made target-specific by attaching specific ligands on the surface. Parenteral use of these novel particles for systemic gene transfer is under development. Recently, reconstituted chylomicron remnants have been used to solubilize DNA-**cationic** **lipid** complexes. This new non-virus vector induces high-level transgene expression in the liver. These formulations were discussed in terms of their efficiency, toxicity and uses in gene therapy. (0 ref)

L13 ANSWER 36 OF 53 CANCERLIT
AN 97616191 CANCERLIT
DN 97616191
TI Efficacy of p53 gene delivered by non-viral carrier against human lung cancer cells (Meeting abstract).
AU Zou Y; Ling Y H; Lozano G; Perez-Soler R
CS University of Texas, MD Anderson Cancer Center, Houston, TX.
SO Proc Annu Meet Am Assoc Cancer Res, (1997). Vol. 38, pp. A64.
ISSN: 0197-016X.
DT (MEETING ABSTRACTS)
FS ICDB
LA English
EM 199708
AB Lung cancer is the result of sequential genetic damage in the bronchial epithelium and the first cause of cancer related death. We have developed **lipid**-based gene delivery systems using a variety of **cationic** lipids for chronic gene replacement therapy in the respiratory tract. The p53 gene was selected to prove feasibility since it is mutated in 30% and 60% of in situ and invasive lung cancers, respectively. The human non small lung carcinoma H358 (p53 null) and H322 (mutant p53) cells were used as in vitro and in vivo targets. The transfection efficiency was highly dependent on the size of the liposomal carrier used and the type of **lipid**/DNA particles formed. Liposomes of 100 **nm** or less in **diameter** result in small size **lipid**/DNA complexes which have the highest transfection efficiency, in the range of 10-12% as determined by beta-galactosidase staining. Under conditions of optimal transfection efficiency, the p53 null cells H358 were found to undergo cell death by apoptosis: 54 hours after a single transfection, 40-50% cells showed morphological signs of apoptosis and DNA fragmentation by agarose gel electrophoresis. The p53 mutant H322 cells were less sensitive to p53 transfection than the p53 null H358 cells, requiring two consecutive transfections to display the same level of cytotoxicity. Both cells were successfully implanted orthotopically into the lungs of nude mice by direct intratracheal inoculation. Therapeutic experiments using multiple intratracheal injections of **lipid**/p53 in nude mice bearing endobronchial H358 and H322 xenografts are in progress.

L13 ANSWER 35 OF 53 MEDLINE DUPLICATE 21

AN 97246097 MEDLINE

DN 97246097 PubMed ID: 9090708

TI Self-assembling DNA-**lipid** particles for gene transfer.

AU Zhang Y P; Reimer D L; Zhang G; Lee P H; Bally M B

CS British Columbia Cancer Agency, Division of Medical Oncology, Vancouver, Canada.

SO PHARMACEUTICAL RESEARCH, (1997 Feb) 14 (2) 190-6.
Journal code: 8406521. ISSN: 0724-8741.

CY United States

DT Journal; Article; (JOURNAL ARTICLE)

LA English

FS Priority Journals

EM 199706

ED Entered STN: 19970630
Last Updated on STN: 19970630
Entered Medline: 19970617

AB PURPOSE: We have demonstrated that a heteromolecular complex consisting of **cationic** lipids and DNA can be prepared and isolated (1). **Cationic** lipids bind DNA through electrostatic interactions. However, when sufficient lipids are bound to DNA the physical and chemical properties of the complex are governed by hydrophobic effects. Here we describe an approach where this hydrophobic complex is used as an intermediate in the preparation of **lipid**-DNA particles (LDPs). METHODS: The approach relies on the generation of mixed micelles containing the detergent, n-octyl beta-D-glucopyranoside (OGP), the **cationic lipid**, N-N-dioleoyl-N, N-dimethylammonium chloride (DODAC), and selected zwitterionic lipids, 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE) or egg sphingomyelin (SM). RESULTS: When these micelles were prepared at low detergent concentrations (20 mM OGP) and combined with pCMV beta DNA, LDPs spontaneously formed. The mean **diameter** of these particles as measured by quasielastic light scattering was 55-70 **nm**, a result that was confirmed by negative stain electron microscopy. Further characterization of these LDPs showed that DNA within the particles was inaccessible to the small fluorochrome TO-PRO-1 and protected against DNase I degradation. LDPs could also be prepared in high concentrations of OGP (100 mM), however particles formed only after removal of OGP by dialysis. Particles formed in this manner were large (> 2000 **nm**) and mediated efficient transfection of Chinese hamster ovary cells. Transfection activity was greater when the **lipid** composition used consisted of SM/ DODAC. Small particles (< 100 **nm**) prepared of SM/DODAC were, however, inefficient transfecting agents. CONCLUSIONS: We believe that LDP formation is a consequence of the molecular forces that promote optimal hydrocarbon-hydrocarbon interactions and elimination of the hydrocarbon-water interface.

L13 ANSWER 32 OF 53 BIOTECHDS COPYRIGHT 2002 THOMSON DERWENT AND ISI
AN 1997-09503 BIOTECHDS
TI Nebulised composition for transfecting lung cells in vivo; DNA cassette and **liposome** formulation for lung cell lipofection and e.g. cystic fibrosis gene therapy
AU Debs R J; Zhu N
PA Univ.California
LO Oakland, CA, USA.
PI US 5641662 24 Jun 1997
AI US 1993-29022 10 Mar 1993
PRAI US 1993-29022 10 Mar 1993
DT Patent
LA English
OS WPI: 1997-340946 [31]

AB A claimed composition comprises a nebulized transfection agent prepared by nebulizing a mixture of a complex of DNA molecules (DNA cassettes and **cationic** lipids in an adjuvant). The **cationic** lipids form vesicles of **diameter** 100-500 **nm** (liposomes) and the DNA cassettes and the **cationic** lipids are present at 4:1 to 1:2 mg DNA to umol **cationic lipid**, where the mixture is free of macroaggregates of the complexes. The expression cassettes comprise a DNA sequence that is transcribed to produce a transcription product in vivo in a mammalian lung cell transfected by the nebulized transfection agent. The DNA sequence comprises an inducible promoter that is cell-specific, tissue-specific or hormone-responsive. Also claimed are: a method for transfecting mammalian lung cells (distal airway or proximal airway cells, preferably tracheal cells or normal lung cells) in vivo and obtaining synthesis of a protein in the cells, where the method involves contacting the cells with the nebulized composition; and transfecting cells lining conducting airways and alveoli involving using the composition. The methods are useful in e.g. cystic fibrosis gene therapy. (58pp)

L13 ANSWER 31 OF 53 MEDLINE DUPLICATE 19

AN 1998122674 MEDLINE

DN 98122674 PubMed ID: 9462842

TI Atomic force microscopy for studying gene transfection mediated by **cationic** liposomes with a **cationic** cholesterol derivative.

AU Kawaura C; Noguchi A; Furuno T; Nakanishi M

CS Faculty of Pharmaceutical Sciences, Nagoya City University, Japan.

SO FEBS LETTERS, (1998 Jan 2) 421 (1) 69-72.
Journal code: 0155157. ISSN: 0014-5793.

CY Netherlands

DT Journal; Article; (JOURNAL ARTICLE)

LA English

FS Priority Journals

EM 199802

ED Entered STN: 19980306
Last Updated on STN: 19980306
Entered Medline: 19980224

AB Atomic force microscopy (AFM) was used for studying gene transfection mediated by **cationic** liposomes which contain a **cationic** cholesterol derivative with a different spacer arm. **Cationic** liposomes were made by a mixture of one of eight **cationic** cholesterol derivatives and 1,2-dioleoyl-sn-glycero-3-phosphatidyl ethanolamine (DOPE). AFM images showed that vesicles made of the **liposome**/DNA complex had various diameters depending on each **cationic** cholesterol derivative with a different spacer arm. The results showed that the **diameter** of the **liposome**/DNA complex was well related to the transfection activity of plasmid pSV2CAT DNA to a cultured cell line (NIH3T3). From the results it was found that the vesicles with moderate diameters (from 0.4 to 1.4 microm) were moste effective for gene transfection of plasmid pSV2CAT DNA into the target cell. Neither smaller vesicles (< 400 **nm**) nor larger vesicles (> 1.4 microm) were adequate for gene transfection. As the gene transfection by the **cationic** liposomes was mostly inhibited by wortmannin, an inhibitor of endocytosis, it is suggested that the vesicles with moderate diameters were useful for gene transfection by endocytosis.

L13 ANSWER 29 OF 53 BIOSIS
AN 1999:258421 BIOSIS
DN PREV199900258421
TI **Lipid**-based systems for the intracellular delivery of genetic drugs.
AU Maurer, Norbert (1); Mori, Atsu; Palmer, Lorne; Monck, Myrna A.; Mok, Kenneth W. C.; Mui, Barbara; Akhong, Quet F.; Cullis, Pieter R.
CS (1) Department of Biochemistry and Molecular Biology, University of British Columbia, 2146 Health Sciences Mall, Vancouver, BC, V6T 1Z3 Canada
SO Molecular Membrane Biology, (Jan.-March, 1998) Vol. 16, No. 1, pp. 129-140.
ISSN: 0968-7688.
DT General Review
LA English
SL English
AB Currently available delivery systems for genetic drugs have limited utility for systemic applications. **Cationic liposome**/ plasmid DNA or oligonucleotide complexes are rapidly cleared from circulation, and the highest levels of activity are observed in 'first pass' organs, such as the lungs, spleen and liver. Engineered viruses can generate an immune response, which compromises transfection resulting from subsequent injections and lack target specificity. A carrier, which can accumulate at sites of diseases such as infections, inflammations and tumours, has to be a small, neutral and highly serum-stable particle, which is not readily recognized by the fixed and free macrophages of the reticuloendothelial system (RES). This review summarizes **lipid** -based technologies for the delivery of nucleic acid-based drugs and introduces a new class of carrier systems, which solve, at least in part, the conflicting demands of circulation longevity and intracellular delivery. Plasmid DNA and oligonucleotides are entrapped into **lipid** particles that contain small amounts of a positively charged **lipid** and are stabilized by the presence of a polyethylene glycol (PEG) coating. These carriers protect nucleic acid-based drugs from degradation by nucleases, are on average 70 **nm** in **diameter**, achieve long circulation lifetimes and are capable of transfecting cells.

L13 ANSWER 25 OF 53 MEDLINE DUPLICATE 16
AN 1999031207 MEDLINE
DN 99031207 PubMed ID: 9813664
TI Characterization of **cationic lipid**-protamine-DNA (LPD) complexes for intravenous gene delivery.
AU Li S; Rizzo M A; Bhattacharya S; Huang L
CS Department of Pharmacology, University of Pittsburgh School of Medicine, PA, USA.
NC CA 59327 (NCI)
CA 64654 (NCI)
DK 44935 (NIDDK)
+
SO GENE THERAPY, (1998 Jul) 5 (7) 930-7.
Journal code: 9421525. ISSN: 0969-7128.
CY ENGLAND: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199811
ED Entered STN: 19990115
Last Updated on STN: 19990115
Entered Medline: 19981130
AB A previous study has shown an efficient, systemic transgene expression in mice via intravenous administration of a LPD formulation composed of DOTAP liposomes, protamine sulfate and plasmid DNA. In this study, factors affecting the in vivo performance of this formulation were further evaluated. A protocol in which liposomes were mixed with protamine before the addition of plasmid DNA was shown to produce small condensed particles with a **diameter** of about 135 **nm**. These particles were stable over time and gave a high level of gene expression in all tissues examined including lung, heart, spleen, liver and kidney with the highest level of expression in the lung. Inclusion of dioleoylphosphatidylethanolamine (DOPE) as a helper **lipid** significantly decreased the in vivo activity of LPD. In contrast, inclusion of cholesterol as a helper **lipid** increased the in vivo transfection efficiency of LPD and more importantly, decrease the amount of **cationic lipid** required for the maximal level of gene expression. Studies on the interaction between mouse serum and LPD showed that LPD became negatively charged after exposure to serum, and LPDs containing different helper lipids varied in the amount of associated serum proteins. LPD containing DOPE was more enriched in a protein corresponding to albumin in molecular weight. These results suggest that the mechanism of LPD-mediated intravenous gene delivery might be different from that of in vitro lipofection and that serum protein association might be a major factor limiting the in vivo transfection by LPD.

L13 ANSWER 23 OF 53 BIOTECHDS COPYRIGHT 2002 THOMSON DERWENT AND ISI

AN 1999-10987 BIOTECHDS

TI Biodistribution and gene expression of **lipid**-plasmid complexes after systemic administration;
in vivo gene transfer efficiency, organ deposition and cellular uptake of **liposome**-plasmid formulations

AU Mahato R I; Anwer K; Tagliaferri F; Meaney C; Leonard P; Wadhwa M S; Logan M; French M; Rolland A

CS GeneMedicine

LO GeneMedicine Inc., 8301 New Trails Drive, The Woodlands, TX 77381-4248, USA.

SO Hum.Gene Ther.; (1998) 9, 14, 2083-99
CODEN: HGTHE3 ISSN: 1043-0342

DT Journal

LA English

AB **Lipid**/plasmid complexes were examined in terms of their physicochemical properties, in vivo gene transfer efficiency, and organ distribution. Plasmids encoding human somatotropin (hST), human Factor-IX (FIX) and chloramphenicol-acetyltransferase (CAT, EC-2.3.1.28) were used. Various combinations of **cationic lipid** to colipid, **lipid**/plasmid ratios, and methods of preparation were compared. Variation of these factors that led, e.g., to alterations in particle size and charge density influenced transfection efficiency after systemic administration of plasmids complexed with **cationic** lipids DOTMA, DOLCE, MMCE, EDOPC and EPMPC. The deposition of DOLCE:DOPE/pCMV-CAT complexes in lung and liver was mainly influenced by the **lipid**/DNA charge ratio, but was relatively insensitive to the amount of colipid contained in the formulation. Significant levels of hST in serum, human FIX in plasma, and CAT in lung and liver were observed with several formulations. Optimal gene expression was generally obtained with low amounts of colipid (cholesterol or DOPE), positively charged **lipid**/plasmid complexes, and liposomes with a **diameter** of at least 200 **nm**. (60 ref)

L13 ANSWER 20 OF 53 MEDLINE DUPLICATE 13

AN 1999363970 MEDLINE

DN 99363970 PubMed ID: 10435112

TI Stabilized plasmid-**lipid** particles: construction and characterization.

AU Wheeler J J; Palmer L; Ossanlou M; MacLachlan I; Graham R W; Zhang Y P; Hope M J; Scherrer P; Cullis P R

CS Inex Pharmaceuticals Corporation, Burnaby, BC, Canada.

SO GENE THERAPY, (1999 Feb) 6 (2) 271-81.
Journal code: 9421525. ISSN: 0969-7128.

CY ENGLAND: United Kingdom

DT Journal; Article; (JOURNAL ARTICLE)

LA English

FS Priority Journals

EM 199908

ED Entered STN: 19990827
Last Updated on STN: 19990827
Entered Medline: 19990817

AB A detergent dialysis procedure is described which allows encapsulation of plasmid DNA within a **lipid** envelope, where the resulting particle is stabilized in aqueous media by the presence of a poly(ethyleneglycol) (PEG) coating. These 'stabilized plasmid-**lipid** particles' (SPLP) exhibit an average size of 70 **nm** in **diameter**, contain one plasmid per particle and fully protect the encapsulated plasmid from digestion by serum nucleases and E. coli DNase I. Encapsulation is a sensitive function of **cationic lipid** content, with maximum entrapment observed at dioleoyldimethylammonium chloride (DODAC) contents of 5 to 10 mol%. The formulation process results in plasmid-trapping efficiencies of up to 70% and permits inclusion of 'fusigenic' lipids such as dioleoylphosphatidylethanolamine (DOPE). The in vitro transfection capabilities of SPLP are demonstrated to be strongly dependent on the length of the acyl chain contained in the ceramide group used to anchor the PEG polymer to the surface of the SPLP. Shorter acyl chain lengths result in a PEG coating which can dissociate from the SPLP surface, transforming the SPLP from a stable particle to a transfection-competent entity. It is suggested that SPLP may have utility as systemic gene delivery systems for gene therapy protocols.

L13 ANSWER 10 OF 53 MEDLINE DUPLICATE 6
AN 2000220615 MEDLINE
DN 20220615 PubMed ID: 10758914
TI Stabilized plasmid-**lipid** particles: pharmacokinetics and plasmid delivery to distal tumors following intravenous injection.
AU Monck M A; Mori A; Lee D; Tam P; Wheeler J J; Cullis P R; Scherrer P
CS Inex Pharmaceuticals Corporation, Burnaby, BC, Canada.
SO JOURNAL OF DRUG TARGETING, (2000) 7 (6) 439-52.
Journal code: 9312476. ISSN: 1061-186X.
CY Switzerland
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200006
ED Entered STN: 20000613
Last Updated on STN: 20000613
Entered Medline: 20000601
AB A previous study has shown that plasmid DNA can be encapsulated in **lipid** particles (SPLP, "stabilized plasmid **lipid** particles") of approximately 70 **nm diameter** composed of 1,2-dioleoyl-3-phosphatidyl-ethanolamine (DOPE), the **cationic lipid** N,N-dioleoyl-N,N-dimethylammonium chloride (DODAC) and poly(ethylene glycol) conjugated to ceramide (PEG-Cer) using a detergent dialysis process (Wheeler et al. (1999) Gene Therapy 6, 271-281). In this work we evaluated the potential of these SPLPs as systemic gene therapy vectors, determining their pharmacokinetics and the biodistribution of the plasmid and **lipid** components. It is shown that the blood clearance and the biodistribution of the SPLPs can be modulated by varying the acyl chain length of the ceramide group used as **lipid** anchor for the PEG polymer. Circulation lifetimes observed for SPLPs with PEG-CerC14 and PEG-CerC20 were t(1/2) = approximately 1 and approximately 10 h, respectively. The SPLPs are stable while circulating in the blood and the encapsulated DNA is fully protected from degradation by serum nucleases. The accelerated clearance of SPLPs with PEG-CerC14 is accompanied by increased accumulation in liver and spleen as compared to PEG-CerC20 SPLPs. Delivery of intact plasmid to liver and spleen was detected. Significant accumulation (approximately 10% of injected dose) of the long circulating SPLPs with PEG-CerC20 in a distal tumor (Lewis lung tumor in the mouse flank) was observed following i.v. application and delivery of intact plasmid to tumor tissue at approximately 6% injected dose/g tissue is demonstrated.

L13 ANSWER 7 OF 53 MEDLINE DUPLICATE 4
AN 2001064791 MEDLINE
DN 20560370 PubMed ID: 11110420
TI Stabilized plasmid-**lipid** particles for systemic gene therapy.
AU Tam P; Monck M; Lee D; Ludkovski O; Leng E C; Clow K; Stark H; Scherrer P; Graham R W; Cullis P R
CS Inex Pharmaceuticals Corporation, Burnaby, BC, Canada.
SO GENE THERAPY, (2000 Nov) 7 (21) 1867-74.
Journal code: 9421525. ISSN: 0969-7128.
CY ENGLAND: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200012
ED Entered STN: 20010322
Last Updated on STN: 20010322
Entered Medline: 20001222
AB The structure of 'stabilized plasmid-**lipid** particles' (SPLP) and their properties as systemic gene therapy vectors has been investigated. We show that SPLP can be visualized employing cryo-electron microscopy to be homogeneous particles of **diameter** 72 +/- 5 **nm** consisting of a **lipid** bilayer surrounding a core of plasmid DNA. It is also shown that SPLP exhibit long circulation lifetimes (circulation half-life >6 h) following intravenous (i.v.) injection in a murine tumor model resulting in accumulation of up to 3% of the total injected dose and concomitant reporter gene expression at a distal (hind flank) tumor site. In contrast, i v. injection of naked plasmid DNA or plasmid DNA-**cationic liposome** complexes did not result in significant plasmid delivery to the tumor site or gene expression at that site. Furthermore, it is shown that high doses of SPLP corresponding to 175 microg plasmid per mouse are nontoxic as assayed by monitoring serum enzyme levels, whereas i.v. injection of complexes give rise to significant toxicity at dose levels above 20 microg plasmid per mouse. It is concluded that SPLP exhibit properties consistent with potential utility as a nontoxic systemic gene therapy vector.

L23 ANSWER 34 OF 34 CAPLUS

AN 1997:349975 CAPLUS

DN 127:23625

TI **Gene** transfer by **liposome**-entrapped polycation-condensed **DNA**: LPDI and LPDII

AU Lee, Robert J.; Huang, Leaf

CS Laboratory of Drug Targeting, Department of Pharmacology, University of Pittsburgh School of Medicine, Pittsburgh, PA, 15261, USA

SO Artificial Self-Assembling Systems for Gene Delivery, developed from Two Conferences, Wakefield, Mass., Sept. 28-29, 1995, and Washington, D. C., Oct. 10-11, 1995 (1996), Meeting Date 1995, 169-176. Editor(s): Felgner, Philip L. Publisher: American Chemical Society, Washington, D. C. CODEN: 64KHA5

DT Conference; General Review

LA English

AB A review with 6 refs. Two novel **gene** transfer vectors have been developed in which **DNA** is first condensed with a polycation and then entrapped into either **cationic** (LPDI) or anionic (LPDII) liposomes by self-assembly. Both LPDI (.apprxeq.52-74 nm in diam.) and LPDII (.apprxeq.70-80 nm) appeared as highly compact, spherical particles when examd. by neg.-stain electron microscopy. LPDI and LPDII with a net pos. charge mediated highly efficient yet non-specific transfection in cultured cells. **Gene** transfer by LPDII carrying a net neg. charge requires a targeting **ligand**. When targeted with **folate**, LPDII mediated receptor-specific transfection in KB cells, a cell line overexpressing the **tumor** marker **folate** receptor. LPDI and LPDII were colloidally stable and more efficient in transfection than **DNA**:DC-chol **cationic** **liposome** complexes in vitro, therefore, show promise as single-vial **gene** therapy vectors.

L23 ANSWER 33 OF 34 CAPLUS

AN 1998:133534 CAPLUS

DN 128:162873

TI **Cationic liposome:DNA** complex vehicles encoding anti-angiogenic peptides for use in **gene** therapy

IN Mixson, Archibald James

PA Mixson, Archibald James, USA

SO Eur. Pat. Appl., 47 pp.
CODEN: EPXXDW

DT Patent

LA English

FAN.CNT 3

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	EP 819758	A2	19980121	EP 1997-112154	19970716
	EP 819758	A3	19980204		
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				
	US 6080728	A	20000627	US 1997-985526	19971205
	JP 11187886	A2	19990713	JP 1998-201996	19980716
PRAI	US 1996-680845	A	19960716		
	EP 1997-112154	A	19970716		
	US 1997-985526	A	19971205		

AB **Cationic** vehicles:**DNA** complexes comprising **DNA** encoding an anti-angiogenic peptide or **DNA** encoding a **tumor** suppressor protein and **DNA** encoding an anti-angiogenic peptide, as well as their use in **gene** therapy, are disclosed. The liposomal components may comprise 1,2-dioleoyl-sn-glycero-3-ethylphosphocholine, 1,2-dimyristoyl-sn-glycero-3-ethylphosphocholine, and 2,3-dioleoyloxy(propyl-N,N,N-trimethylammonium chloride), optionally in combination with polyethylene glycol and a targeted **ligand** such as Arg-Gly-Asp, ferritin, or antibodies targeted toward HER2. **DNA** is prepd. encoding anti-angiogenic peptide fragments of thrombospondin I, fibronectin, laminin, platelet factor 4, angiostatin, and prolactin, as well as concatamers of these fragments. **Tumor** suppressor protein genes include p53, p21, or Rb. Thus, **liposome:DNA** vectors encoding p53 in combination with a thrombospondin I fragment reduced tumors more effectively than p53 alone. The **cationic** polymer allows superior transfection of endothelial cells; Superfect is a better transfection agent than **cationic** liposomes for many different cell lines.

L23 ANSWER 30 OF 34 CAPLUS COPYRIGHT 2002 ACS
AN 2000:229505 CAPLUS
DN 133:79105
TI Drug delivery system for **cancer gene** therapy -a review
AU Niitsu, Yoshiro; Takahashi, Minoru; Sato, Yasushi
CS Fourth Deparment of Internal Medicine, Sapporo Medica l University School of Medicine, Chuo-ku, Sapporo, 060-8543, Japan
SO Drug Delivery System (2000), 15(1), 32-38
CODEN: DDSYEI; ISSN: 0913-5006
PB Nippon DDS Gakkai Jimukyoku
DT Journal; General Review
LA Japanese
AB Drug delivery system (DDS), currently employed for **cancer gene** therapy was reviewed with 13 refs. There have been at least four systems hitherto developed to deliver the anticancer genes to **tumor**. They include 1) modified **liposome** system such as **cationic liposome**, HVJ-**liposome**, transferrin conjugated **liposome** etc. 2) naked **plasmid DNA** conjugated with **ligand** or antibody which are known to interact with receptor or antigen on **tumor** cells. 3) viral vectors such as retroviral vector, adenoviral vector, lentiviral vector, adeno-assocd. viral vector etc. 4)replication competent oncolytic viruses such as E1B attenuated adenovirus or genetically modified herpes simplex virus. Although most of these delivery systems are found to be applicable for local administration of transgene, little success has been reported as to specific targeting to **tumor** by systemic administration. Therefore efforts are now focusing on the development of devise to target **tumor** in vivo.

L23 ANSWER 28 OF 34 EMBASE

AN 1999287253 EMBASE

TI Systemic p53 **gene** therapy in combination with radiation results in human **tumor** regression.

AU Xu L.; Pirollol K.F.; Rait A.; Murray A.L.; Chang E.H.

CS Dr. E.H. Chang, Lombardi Cancer Center, Research Building/FA20, Georgetown University Medical Center, 3970 Reservoir Road, NW, Washington, DC 20007, United States. change@gunet.georgetown.edu

SO Tumor Targeting, (1999) 4/2 (92-104).
Refs: 38
ISSN: 1351-8488 CODEN: TUTAF

CY United Kingdom

DT Journal; Article

FS 016 Cancer
037 Drug Literature Index

LA English

SL English

AB A long-standing goal in **gene** therapy for **cancer** is a systemic delivery system that selectively targets **tumor** cells including metastases. We optimized a **folate** containing **cationic liposome** system for the systemic delivery of wtp53 to squamous cell carcinoma of the head and neck. The **folate ligand**, which serves to target the complex to **tumor** cells, increased the transfection efficiency by facilitating transient **gene** transfection. This system was demonstrated to be exceedingly **tumor**-selective in that normal tissues, including the highly proliferative gut and bone marrow, were not transfected. The systemic delivery by this method of wild-type p53 to established mouse xenografts markedly sensitized these human tumors to radiotherapy. This combination of systemic p53 **gene** therapy and conventional radiotherapy resulted in complete **tumor** regression and inhibition of their recurrence long-term. Similar results were also demonstrated with another model system, prostate **cancer** cell line DU145. This addition of a molecular component could provide an improved therapeutic approach for

site-specific. (20pp)

L23 ANSWER 23 OF 34 BIOTECHDS COPYRIGHT 2002 THOMSON DERWENT AND ISI
AN 1996-11678 BIOTECHDS
TI Use of targeted **cationic** liposomes in enhanced **DNA** delivery to **cancer** cells;
beta-galactosidase reporter **gene** expression in HCT-15 and HepG2 cell culture by lipofection, for application in **cancer** **gene** therapy
AU Kao G Y; Chang L J; *Allen T M
CS Univ.Alberta
LO Departments of Pharmacology, University of Alberta, Edmonton, Alberta, AB T6G 2H7, Canada.
SO Cancer Gene Ther.; (1996) 3, 4, 250-56
CODEN: 2815V ISSN: 0929-1903
DT Journal
LA English
AB Targeted delivery to **cancer** cells in vitro of a **plasmid** containing the beta-galactosidase (EC-3.2.1.23) reporter **gene** (beta-gal) using a **cationic liposome** formulation composed of DOTAP and DOPE is reported. HCT-15 and HepG2 cells were plated at 20,000-80,000 cells/well in a 96-well plate. On the 2nd day, equal volumes of **cationic lipid** solution (0.6-1 mM) and **DNA** solution (40-160 ug/ml) containing a beta-gal reporter **plasmid** pSp72CMV-NuLacZpA were mixed to form **lipid-DNA** complexes. These were incubated within 0.5-1 hr with the cells. All cells were incubated with the **DNA-lipid** complex at 37 deg in a humidified incubator with 5% CO2 air for 4-6 hr in the absence of fetal cattle serum (FCS). The FCS was then added to a concentration of 10% for the remainder of the incubation time. Notable increases in expression of the beta-gal reporter **gene** were observed in vitro in monoclonal antibody-targeted liposomes, compared with non-targeted liposomes, in both cell lines. Use of asialofetuin as a targeting **ligand** significantly increased expression of the reporter **gene** in human hepatoma cells. (30 ref)

L23 ANSWER 22 OF 34 BIOTECHDS COPYRIGHT 2002 THOMSON DERWENT AND ISI
AN 1997-11225 BIOTECHDS
TI Non-viral cell-specific vector for **gene** transfer containing targeting **ligand**;
for use in **gene** therapy
AU Sedlacek H H; Klenk H D; Kissel T; Mueller R
PA Hoechst
LO Frankfurt, Germany.
PI DE 19605279 14 Aug 1997
AI DE 1996-1005279 13 Feb 1996
PRAI DE 1996-1005279 13 Feb 1996
DT Patent
LA German
OS WPI: 1997-403849 [38]
AB A new cell-specific expression vector, preferably a **plasmid**, for introduction of a target **gene** into the cells of an organism, especially for use in **gene** therapy, contains a non-viral adsorbent (e.g. a protein, phospholipid, **cationic lipid**, glycoprotein, lipoprotein or lipopolyamine, preferably **cationic** or lipophilic, especially albumin or xylan), a **ligand** that binds specifically to target cells (e.g. an antibody, cytokine, growth factor, viral envelope glycoprotein or mannose-containing glycoprotein), a fusion protein for penetration into target cell cytoplasm (e.g. hemagglutinin of influenza A or B virus, M2 protein of influenza A virus or transmembrane glycoprotein of filovirus or rabies virus) and the target **gene**. A specific use is expression of beta-glucuronidase (EC-3.2.1.31) in proliferating endothelial cells for conversion of beta-glucuronide precursors to active antiproliferative or cytostatic drugs, able to inhibit cell proliferation, **tumor** growth and local inflammatory reactions. These constructs do not cause mutations in the host genome and are site-specific. (20pp)

L23 ANSWER 21 OF 34 BIOTECHDS COPYRIGHT 2002 THOMSON DERWENT AND ISI
AN 1998-07984 BIOTECHDS
TI **Ligand** system for specific **gene** transfer of
gene construct to target cells;
target cell-specific **ligand,** e.g. growth factor and
gene construct-specific **ligand,** e.g. human
double-chain Fv fusion protein preparation, used for disease
gene therapy
AU Sedlacek H H; Mueller R
PA Hoechst
LO Frankfurt, Germany.
PI DE 19649645 4 Jun 1998
AI DE 1996-1049645 29 Nov 1996
PRAI DE 1996-1049645 29 Nov 1996
DT Patent
LA German
OS WPI: 1998-313488 [28]
AB A new **ligand** system for target cell-specific transfer of **DNA** sequences consists of at least one linker and at least one **gene** construct-specific **ligand,** where the **gene** construct-specific **ligand** is an antibody or fragment (especially a human F(ab)2, double-chain Fv, single-chain Fv or Fc fragment) that binds directly or indirectly to the **gene** construct. A preferred system further consists of a target cell-specific **ligand** which is preferably a growth factor, cytokine, interferon, **tumor** necrosis factor, chemokine, peptide hormone, angiotensin, kinin, histamine, steroid, adhesion molecule or vitamin which binds to the surface of a target cell. A preferred **gene** construct consists of naked **DNA,** naked RNA or a **plasmid,** optionally mixed with a **cationic** polymer, peptide, protein, **lipid** or phospholipid. Also claimed is a **ligand** system mixed with a **gene** construct. The new **ligand** system mixed with the **gene** construct may be administered locally or by injection for therapy or prevention of diseases of the skin, mucosa, nervous system, internal organs, blood clotting system, hematopoietic system, immune system, musculature, connective tissue or joints. (17pp)

L23 ANSWER 20 OF 34 BIOTECHDS COPYRIGHT 2002 THOMSON DERWENT AND ISI
AN 1998-11332 BIOTECHDS
TI Peptide, **nucleic** acid and agent composition which enhances transfection;
where the peptide is linked to the **nucleic** acid or transfection agent for enhanced transfection efficiency, **gene** therapy, etc.
AU Hawley-Nelson P; Lan J; Shih P; Jessee J A; Ciccarone V C; Evans K L; Schifferli K P; Gebeyehu G
PA Life-Technol.
LO Rockville, MD, USA.
PI WO 9840502 17 Sep 1998
AI WO 1998-US5232 16 Mar 1998
PRAI US 1997-818200 14 Mar 1997
DT Patent
LA English
OS WPI: 1998-520821 [43]
AB A composition (I) is claimed for transfecting a cell comprising a **nucleic** acid (NA), a peptide or protein (PP) and a transfection agent (TA). Also claimed are peptides comprising a NLS sequence, or a Tat sequence, modified by covalent bonding to a NA-binding group. (I) can be used in therapeutic, diagnostic and research methods. Transfection reagent kits are provided. PP or modified PPs result in enhanced transfection. (I) can be used for intra- or extracellular delivery or targeting of cells for the diagnosis and treatment of diseases, **gene** therapy, viral inhibition, and for the introduction of antisense or antigenic NAs. (I) is especially useful in **cancer** treatment. Preferably (I) has 2 TAs which are monovalent **cationic** lipids, neutral lipids (e.g. cholesterol) or dendrimers. Preferably the PP is a nuclear localization PP, a fusion protein, a receptor-**ligand** PP, a transport PP or is involved in cell adhesion, and may be viral (e.g. adeno virus, hepatitis virus, etc) or insulin, transferrin, or one of 33 other specified PPs. (I) can transfect animal or human cells (preferably fibroblasts), a primary cell culture, a passaged cell culture or a cell line. (103pp)

L23 ANSWER 19 OF 34 BIOTECHDS COPYRIGHT 2002 THOMSON DERWENT AND ISI

AN 1999-14611 BIOTECHDS

TI In vivo **gene** transfer by targeted non-viral **gene** delivery;
using ligands and molecular biology to increase selective delivery and expression to **tumor** vasculature (conference abstract)

AU Sullivan S M; Anwer K; Szymanski P; Kao G; Shelvin B; Xiang Q

CS GeneMedicine

LO GeneMedicine, 8301 New Trails Drive, The Woodlands, TX 77381, USA.

SO Abstr.Pap.Am.Chem.Soc.; (1999) 217 Meet., Pt.2, POLY149
CODEN: ACSRAL ISSN: 0065-7727
217th ACS National Meeting, American Chemical Society, Anaheim, CA, USA, 21-25 March, 1999.

DT Journal

LA English

AB Targeting strategies for non-viral **gene** therapy were represented. The focus was on systemic administration for transfection of **tumor** endothelium. The **gene** delivery technology used a combination of ligands and molecular biology to increase selective delivery and expression to **tumor** vasculature. Integrin binding peptides were used as targeting ligands for **cationic lipid** based transfection complexes. The peptides were first screened for **tumor** uptake using radiolabeled liposomes systemically administered to mice with subcutaneous primary tumors. The other targeting aspect was the expression system. Expression plasmids were developed containing promoters that yield selective expression in proliferating cells. The combination of the **ligand** targeted system with tissue selective expression should allow controlled systemic administration of non-viral transfection systems to be developed for **cancer gene** therapy. (0 ref)

L23 ANSWER 10 OF 34 MEDLINE
AN 2000032019 MEDLINE
DN 20032019 PubMed ID: 10564057
TI Optimization of **folate**-conjugated liposomal vectors for **folate** receptor-mediated **gene** therapy.
AU Reddy J A; Dean D; Kennedy M D; Low P S
CS Department of Chemistry, 1393 Brown Building, Purdue University, West Lafayette, Indiana 47907-1393, USA.
SO JOURNAL OF PHARMACEUTICAL SCIENCES, (1999 Nov) 88 (11) 1112-8.
Journal code: 2985195R. ISSN: 0022-3549.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199912
ED Entered STN: 20000113
Last Updated on STN: 20000113
Entered Medline: 19991215
AB A **folate**-targeted transfection complex that is internalized by certain **cancer** cells and displays several properties reminiscent of enveloped viruses has been developed. These liposomal vectors are comprised of a polycation-condensed **DNA plasmid** associated with a mixture of neutral and anionic lipids supplemented with **folate**-poly(ethylene glycol)-dioleylphosphatidylethanolamine for **tumor** cell-specific targeting. N-Citraconyl-dioleylphosphatidylethanolamine is also included for pH-dependent release of endosome-entrapped **DNA** into the cytoplasm, and a novel **plasmid** containing a 366-bp segment from SV40 **DNA** has been employed to facilitate transport of the **plasmid** into the nucleus. Because formation of the **DNA** core is an important step in the assembly of liposomal vectors, considerable effort was devoted to comparing the transfection efficiencies of various **DNA** condensing agents. It was found that complexation of **plasmid DNA** with high molecular weight polymers such as acylated-polylysine and **cationic** dendrimers leads to higher **folate**-mediated transfection efficiency than **DNA** complexed with unmodified polylysine. In contrast, compaction of **plasmid DNA** with small **cationic** molecules such as spermine, spermidine, or gramicidin S yields only weakly active **folate**-targeted liposomal vectors. Compared to analogous liposomal vector preparations lacking an optimally compacted **DNA** core, a cell-specific targeting **ligand**, a caged fusogenic **lipid**, and a nucleotide sequence that facilitates nuclear uptake, these modified liposomal vectors display greatly improved transfection efficiencies and target cell specificity.

L23 ANSWER 9 OF 34 MEDLINE
AN 2000075746 MEDLINE
DN 20075746 PubMed ID: 10609655
TI Transferrin-**liposome**-mediated systemic p53 **gene** therapy in combination with radiation results in regression of human head and neck **cancer** xenografts.
AU Xu L; Pirollo K F; Tang W H; Rait A; Chang E H
CS Department of Otolaryngology, Head and Neck Surgery, Lombardi Cancer Center, Georgetown University Medical Center, Washington, DC 20007, USA.
NC R01 CA 45158 (NCI)
SO HUMAN GENE THERAPY, (1999 Dec 10) 10 (18) 2941-52.
Journal code: 9008950. ISSN: 1043-0342.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200001
ED Entered STN: 20000114
Last Updated on STN: 20000114
Entered Medline: 20000106
AB The use of **cationic** liposomes as nonviral vehicles for the delivery of therapeutic molecules is becoming increasingly prevalent in the field of **gene** therapy. We have previously demonstrated that the use of the transferrin **ligand** (Tf) to target a **cationic liposome** delivery system resulted in a significant increase in the transfection efficiency of the complex [Xu, L., Pirollo, K.F., and Chang, E.H. (1997). Hum. **Gene** Ther. 8, 467-475]. Delivery of wild-type (wt) p53 to a radiation-resistant squamous cell carcinoma of the head and neck (SCCHN) cell line via this **ligand**-targeted, **liposome** complex was also able to revert the radiation resistant phenotype of these cells in vitro. Here we optimized the Tf/**liposome/DNA** ratio of the complex (LipT) for maximum **tumor** cell targeting, even in the presence of serum. The efficient reestablishment of wtp53 function in these SCCHN **tumor** cells in vitro, via the LipT complex, restored the apoptotic pathway, resulting in a significant increase in radiation-induced apoptosis that was directly proportional to the level of exogenous wtp53 in the **tumor** cells. More significantly, intravenous administration of LipT-p53 markedly sensitized established SCCHN nude mouse xenograft tumors to radiotherapy. The combination of systemic LipT-p53 **gene** therapy and radiation resulted in complete **tumor** regression and inhibition of their recurrence even 6 months after the end of all treatment. These results indicate that this **tumor**-specific, **ligand-liposome** delivery system for p53 **gene** therapy, when used in concert with conventional radiotherapy, can provide a new and more effective means of **cancer** treatment.

L23 ANSWER 7 OF 34 MEDLINE
AN 2000297971 MEDLINE
DN 20297971 PubMed ID: 10841276
TI Successful transfection of lymphocytes by ternary lipoplexes.
AU Simoes S; Slepushkin V; Gaspar R; Pedroso de Lima M C; Duzgunes N
CS Department of Microbiology, School of Dentistry, University of the Pacific, San Francisco, CA 94115, USA.
NC AI 35231 (NIAID)
SO BIOSCIENCE REPORTS, (1999 Dec) 19 (6) 601-9.
Journal code: 8102797. ISSN: 0144-8463.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals; AIDS
EM 200007
ED Entered STN: 20000810
Last Updated on STN: 20000810
Entered Medline: 20000724
AB Transgene expression in lymphoid cells may be useful for modulating immune responses in, and **gene** therapy of, **cancer** and AIDS. Although **cationic liposome-DNA** complexes (lipoplexes) present advantages over viral vectors, they have low transfection efficiency, unfavorable features for intravenous administration, and lack of target cell specificity. The use of a targeting **ligand** (transferrin), or an endosome-disrupting peptide, in ternary complexes with liposomes and a luciferase **plasmid**, significantly promoted transgene expression in several T- and B-lymphocytic cell lines. The highest levels of luciferase activity were obtained at a **lipid/DNA** (+/-) charge ratio of 1/1, where the ternary complexes were net negatively charged. The use of such negatively charged ternary complexes may alleviate some of the drawbacks of highly positively charged plain lipoplexes for **gene** delivery.